IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	SRIVASTAVA *et al.*	Confirmation No.:	3088
Serial No.:	09/393,652	Art Unit:	1644
Filed:	September 10, 1999	Examiner:	Gerald R. Ewoldt, Ph.D.
For:	METHODS AND COMPOSITIONS FOR THE TREATMENT AND PREVENTION OF GRAFT REJECTION USING HEAT SHOCK PROTEINS	Attorney Docket No.:	8449-025-999

DECLARATION OF DR. PRAMOD K. SRIVASTAVA UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, PRAMOD K. SRIVASTAVA, do declare and state as follows:

1. I am a citizen of India and a permanent resident of the United States, residing at 70 Pheasant Run, Avon, Connecticut 06001.

2. I am a co-inventor of the invention described and claimed in the above-identified patent application no. 09/393,652 ("the '652 application"). I am a co-founder of Antigenics, Inc., exclusive licensee of the above-identified application.

3. I am the inventor of the invention described and claimed in U.S. Patent No. 5,837,251 ("the '251 patent") which issued November 17, 1998, and which is entitled: "Compositions and Methods Using Complexes of Heat Shock Proteins and Antigenic Molecules for the Treatment and Prevention of Neoplastic Diseases." A copy of the '251 patent is attached as Exhibit C.

4. I am currently Professor of Immunology and Director of the Center for Immunotherapy of Cancer and Infectious Diseases at the University of Connecticut School

of Medicine, the position I have held from January 1997 to the present. From 1993 to December 1996, I was a member of the Department of Biological Sciences at Fordham University, Bronx, New York, where I served as Professor and Head of the Cancer Immunology Program. Fordham University is the assignee of the above-captioned application.

5. My academic and technical experience, honors, and a list of my publications are set forth in my *curriculum vitae*, attached hereto as Exhibit A.

6. I have reviewed and I am familiar with the '652 application. The '652 application teaches the administration of heat shock proteins and non-covalent heat shock protein - peptide complexes for the inhibition of graft rejection.

7. I have reviewed and I am familiar with International Publication number WO 02/072133, which is entitled: "Immunomodulatory Properties of BiP" (hereafter "the '133 publication"). A copy of the '133 publication is attached hereto as Exhibit E.

8. I have reviewed and I am familiar with the pending claims in the '652 application and with the Office Action mailed February 25, 2004 in connection with the '652 application. I have been informed and believe that the claims of the '652 application are rejected for lack of enablement based, in part, upon a contention that the nature of the invention is highly unexpected and therefore the claimed methods are unpredictable and therefore are not enabled for any hsp not demonstrated in the specification or art to be immunosuppressive in the context of graft rejection. I have also been informed and believe that the claims of the '652 application are rejected based, in part, upon a contention that the specific dose ranges of non-covalent heat shock protein - peptide complexes that are disclosed and claimed in the '652 application are too low to be effective, since extrapolation from dosages used in mice by using the same per kg dosage in humans yields a dosage figure much higher than the specific dosage ranges disclosed in the specification.

9. In the paragraphs below, I first discuss the evidence supporting the use of hsp90 family members, such as gp96, and hsp70 family members, such as hsp70, for the inhibition of graft rejection. I will then address the issue raised by the Examiner regarding appropriate dose ranges for heat shock protein complexes used for the inhibition of graft rejection.

10. The experiments described in paragraphs 11 and 12 below demonstrate that gp96 has an immunosuppressive property. Accordingly the data discussed below are supportive of the utility of gp96 for the inhibition of graft rejection. These experiments were conducted by me or by my co-inventor, Dr. Chandawarkar, or by others under my supervision or that of Dr. Chandawarkar, at the Center for Immunotherapy of Cancer and Infectious Diseases, at the University of Connecticut School of Medicine. The experiments described in paragraphs 11 and 12 have been published (Exhibit B).

11. The first experiment demonstrated that gp96 complexes will suppress an immune response directed toward a tumor. In this experiment, BALB/cJ mice were injected, *inter alia*, with either 10 μg of gp96 complexes isolated from Meth A tumor tissue or with a combination of 10 μg of gp96 complexes isolated from Meth A tumor tissue and 90 μg of gp96 complexes isolated from liver. The immunized animals were subsequently challenged by inoculation with live Meth A tumor cells and the growth of the tumor followed for approximately three weeks. Mice immunized with 10 μg of gp96 complexes isolated from Meth A tumor tissue rejected the tumor in four of the five animals tested. However, those mice immunized with the mixture of 10 μg of gp96 complexes isolated from Meth A tumor tissue and 90 μg of gp96 complexes isolated from liver were the same as the buffer control; *i.e.* there was no inhibition of tumor growth (*see e.g.* Fig. 1 of Exhibit B). Thus administration of a total dose of 100 μg of gp96 complexes (10 μg of gp96 complexes isolated from Meth A tumor tissue and 90 μg of gp96 complexes isolated from liver) appears to have suppressed the anti-tumor immune response that had been observed upon administration of 10 μg of gp96 complexes isolated from Meth A tumor tissue.

12. A second experiment demonstrated that gp96 complexes will suppress development of diabetes in NOD mice that develop this disease spontaneously. Administration of 100 μg of gp96 complexes to NOD mice prevented development of diabetes in 60% - 80% of those animals. In addition, these mice remained free of this disease during the entire observation period (*i.e.* for at least six months), as illustrated in Fig. 4(b) of Exhibit B. Moreover, adoptive transfer experiments have established that this suppression of diabetes is mediated by $CD4^+$ T cells (*see e.g.* Exhibit B, at page 619, right column first full paragraph). Administration of 10 μg of gp96 complexes did not inhibit development of diabetes. Thus administration of 100 μg of gp96 complexes inhibited development of an autoimmune disease, diabetes, in NOD mice. These results were obtained regardless of the source from which the gp96 complexes were isolated, and the observed immunosuppression

apparently is a result of the generation of suppressor $CD4^+$ T cells that are able to inhibit a wide variety of specific $CD8^+$ T cell - mediated immune responses.

13. Therefore, these results establish that relatively high doses of gp96 complexes have an immunosuppressive activity. Additionally, experiments described in the '652 application show that administration of 100 µg and 200 µg of gp96 complexes isolated from liver, effectively inhibited rejection of skin grafts in mice (*see* Examples 1 and 2 of the '652 application). Accordingly, in my judgment, this evidence, taken together with the experiments described above, indicates that gp96 complexes are useful for inhibiting graft rejection. More specifically, these data demonstrate that administration of gp96 complexes will inhibit an immune response directed against foreign tissue (*i.e.* Meth A tumor cells) as well as an autoimmune response directed against self tissue, and will also inhibit the immune response underlying rejection of a graft.

14. The evidence also indicates that graft rejection can be inhibited by administration of gp96 complexes, regardless of the species from which those complexes are isolated. Although the experiments described above involved the administration of murine gp96 complexes, it is obvious to me, as it would be to those skilled in this field of research, that such gp96 complexes could be substituted with gp96 complexes isolated from any species, absent a remarkably-unexpected specific demonstration or fact indicating otherwise. This is so because (a) the immunosuppressive effects seen were not dependent on the tissue used for isolation of the gp96 complexes used (*e.g.* tumor, pancreatic, or liver tissue) indicating that the effects are specific to the hsp *per se*, and (b) gp96 proteins are very highly conserved among different species, exhibiting, for example, a minimum of 95% amino acid sequence identity among human, rat, and murine gp96 species (Exhibit D).

15. Even more broadly, the evidence leads me to conclude that graft rejection can be inhibited by administration of heat shock protein complexes wherein the heat shock protein is a member of the hsp90 family of heat shock proteins. This is so because (a) members of an hsp family, *e.g.* the hsp90 family, are expected to have very similar activities, even though different members of an hsp family may comprise amino acid sequence variations (*e.g.* epitopes) that may permit immunological distinctions to be made (*see e.g.* Lindquist *et al.* (1988) "The Heat-Shock Proteins" *Annu. Rev. Genet.* 22: 631-77; particularly 634-40; Exhibit I), (b) the immunosuppressive effects observed were not dependent on the tissue used for isolation of the gp96 complexes used (*e.g.* tumor, pancreatic, or liver tissue) indicating that the effects are specific to the hsp *per se*, and (c) members of the

hsp90 family of heat shock protcins, including hsp90 and gp96, are highly conserved protcins (*see e.g.* Exhibit I, at pages 634-635). For example, there is a minimum of 98% amino acid sequence identity among human, rat, and murine hsp90 species, as well as a minimum of 47% amino acid sequence identity between *e.g.* human gp96 and hsp90 of rat, murine, and human species (Exhibit D). Thus, where one member of a given heat shock protein family has been identified as having a specific activity, such as the ability to inhibit graft rejection, it would be predictable that other members of the same hsp family would have the same activity. Therefore, in view of the disclosure of the '652 application regarding the activity of gp96 complexes in inhibiting graft rejection, it would be expected that members of the hsp90 family would be interchangeable with one another in the claimed methods.

16. Regarding the hsp70 family of heat shock proteins, BiP is a member of the hsp70 family. The '133 publication discloses that the heat shock protein BiP causes $CD14^+$ cells to release IL-10; stimulates $CD8^+$ cells to prolifcrate and release IL-10; inhibits the recall antigen response; and activates the expression of an array of anti-inflammatory genes in monocytes, including the migration inhibitory factor (MIF), the soluble TNF receptor II and TIMPs, which are tissue inhibitor matrix metalloproteinases. The '133 publication also discloses, at page 22, lines 6-24 and in Fig. 9, suppression of the allogeneic response by peripheral blood mononuclear cells (PBMC) when BiP is added at the start of monocyte maturation. This assay is a classic method for measuring the immune response to non-self antigens. Accordingly, these activities of BiP are, collectively, indicative of the use of BiP for inhibiting graft rejection. As I have noted above, once a given member of a heat shock protcin family has been shown to have an activity, it is predictable that the other members of the same heat shock protein family will also have that activity due to intrafamily conservation of structure (*see e.g.* Gething *et al.* (1992) "Protein Folding in the Cell" *Nature* 355: 33-45, esp. page 39, right column through the end of page 40) (Exhibit H). Therefore, since the evidence is supportive of the use of BiP to inhibit graft rejection, the evidence also is supportivo of such use of other members of the hsp70 family.

17. With respect to the use of heat shock protein complexes for *stimulating* an immune response against a non-covalently bound peptide, evidence has shown that the extrapolation of an appropriate dose in humans based upon data obtained in rodents is not directly proportional to their difference in mass. In fact, as indicated for example in U.S. Patent No. 5,837,251, the human dose is approximately the same as that for a mouse, within a factor of fifty. This indication has been demonstrated to be accurate based upon human

clinical trials in which clinical responses have been observed upon administration of doses of gp96 complexes of between 2.5 μg and 100 μg (*see e.g.* Exhibit F (immunization of patients with 25 μg of tumor-derived gp96 complexes) and Exhibit G (immunization of patients with either 5 μg or 50 μg of tumor-derived gp96 complexes)). I am not aware of any information or any reason that would suggest that this minimal impact of weight upon effective dose would not also be applicable with respect to the effective dose of heat shock protein complexes to be administered for graft rejection. Accordingly, I am not aware of any reason why the dose ranges disclosed and claimed in the '652 application would not be appropriate for inhibition of graft rejection in humans.

18. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: Apr 25/04

PRAMOD K. SRIVASTAVA

Attachments:

Exhibit A: *Curriculum vitae* of Pramod K. Srivastava

Exhibit B: Chandawarkar *et al.* (2004) "Immune modulation with high-dose heat shock protein gp96: therapy of murine autoimmune diabetes and encephalomyclitis" *International Immunology* 16(4): 615-624.

Exhibit C: U.S. Patent No. 5,837,251

Exhibit D: Amino acid sequence comparisons between and among human, rat, and mouse gp96 and hsp90 proteins.

Exhibit E: WO 02/072133, "Immunomodulatory Properties of BiP," published September 19, 2002.

Exhibit F: Janetzki *et al.* (2000) "Immunization of Cancer Patients with Autologous Cancer-Derived Heat Shock Protein gp96 Preparations: A Pilot Study, *Int. J. Cancer* 88: 232-238.

Exhibit G: Belli *et al.* (2002) "Vaccination of Metastatic Melanoma Patients With Autologous Tumor-Derived Heat Shock Protein gp96-Peptide Complexes: Clinical and Immunologic Findings *J. Clin. Oncol.* 20: 4169-4180.

Exhibit H: Gething *et al.* (1992) "Protein Folding in the Cell" *Nature* 355: 33-45.

Exhibit I Lindquist *et al.* (1988) "The Heat-Shock Proteins" *Annu. Rev. Genet.* 22: 631-77.

EXHIBIT A

Curriculum Vitae of *Pramod Srivastava*

Contents

Name: Pramod Kumar SRIVASTAVA

Date of Birth: August 16, 1955

Social Security #: 043-74-4345

Home address: 70 Pheasant Run, Avon, CT 06001

Office Address: Center for Immunotherapy of Cancer & Infectious Diseases
University of Connecticut School of Medicine, MC1601
Farmington, CT 06030 - 1601

Tel: 860 679 4444, Fax: 860 679 7905
E-mail: srivastava@nso2.uchc.edu

Education:

1973, B Sc (Zoology, Botany and Chemistry), Allahabad Univ, Allahabad, India, High Second div.;
1975, M Sc (Botany, with specialization in Palaentology, Advisor : Professor Divya Darshan Pant), Allahabad University, Allahabad, India, First Division with First Rank;
1979, Post-Graduate Diploma (Microbiology & Biotechnology; specialization in yeast genetics, Advisor : Professor Yasuji Oshima), Osaka University, Osaka, Japan;
1984, Ph D (Biochemistry with specialization in tumor immunology, Advisor : Dr. M. Ramchandra Das), Osmania Univ. (Centre for Cellular & Molecular Biology), Hyderabad, India.
2006 (Class of) , MD, University of Connecticut, Farmington, CT

Postdoctoral Training:

Postdoctoral Fellow, Yale University, New Haven, Connecticut, 1982-1983, Advisor: Professor Alan Garen
Research Fellow, Sloan-Kettering Institute for Cancer Research, New York, 1984-85, Advisor: Professor Lloyd J. Old

Positions and Appointments:

Scientist 'C', Center for Cellular & Molecular Biology (CSIR), Hyderabad, India	06.83 - 02.84
Research Associate, Sloan-Kettering Institute for Cancer Research, Immunology Program, New York	01.86 - 10.88
Assistant Professor, Department of Pharmacology, Mount Sinai School of Medicine, New York. Also appointed in the Dept of Microbiology	11.88 - 08.93
Associate Professor of Immunology, Dept of Biological Sciences, Fordham University, Bronx, NY.	09.93- 01.96
Professor of Immunology, Department of Biological Sciences, Fordham University, Bronx, NY.	02.96 - 12.96
Professor of Immunology, Physicians Health Service Chair in Cancer Immunology, and Director, Center for Immunotherapy of Cancer and Infectious Diseases, University of Connecticut, School of Medicine, Farmington, CT.	01.97 - now
Director, University of Connecticut Cancer Center,	01.01 – 9.03

Editorial Positions:

Associate Editor, *Human Vaccines*, 2005 -
Associate Editor, *Journal of Immunology*, 2003 - 2005
Editorial Board, *Tissue Antigens*, 2001-
Section Editor, Inflammation and Immunity Section, *Cell Stress and Chaperones,* 2000 -
Editorial Board, *Cellular Immunology,* 2000 - 2004
Editor, *Cancer Immunity,* 2000 -

Membership of Study Sections and Advisory Councils:

AdHoc member of several NIH Study Sections 2-3 times/year, presently;
Experimental Immunology study section, NIH, 1994 - 1999;
Scientific Advisory Council, Cancer Research Institute, New York, and Member, Fellowships Committee of the CRI, 1996 - present;
Chairman, Scientific Advisory Board, and member, Board of Directors, Antigenics, New York, 1995 – present;
Howard Hughes Medical Institute Student Fellowships Review Panel, 1999 – 2001.

Present Grant Support:

NIH/NCI — 7/1/00-6/30/08
5R01CA84479-02 — $225,000 direct cost/year
Receptor for gp96 on Macrophages and Dendritic Cells

Antigenics, L.L.C. — 2/12/98-2/11/04
Use of Heat Shock Proteins for the Development of Therapeutic and Prophylactic Vaccines — $ ~750,000 direct cost/year

Lea's Foundation for Leukemia Research — 9/1/99-12/31/02
Treatment of indolent B-cell lymphoma and CLL patients with heat shock protein 70 (HSP70) complexed to autologous tumor proteins or to tumor-specific proteins — $15,000 direct cost/year

The Charles Dana Foundation — 12/01/01-11/30/05
Harnessing CD91 ligands for rapid treatments of Infections of unknown antigenicity — $100,000 direct cost / year

Peer Reviewed Past Grant Support (selected list):

NIH 5RO1-CA44786-09
Heat Shock Proteins as Tumor Antigens

Peer Reviewed Past Grant Support (selected list): (continued)

NIH 7RO1-CA64394-04
Use of HSP70-Peptide Complexes in

Specific Immunity

NIH R03 CA54990
Immunity to spontaneous cancers of ras- and
jun transgenic mice

NIH 5 P50 CA62924-06
John Hopkins Oncology Center
Influence of Random Mutations on the
Antigenicity of Colorectal Tumors

DARPA (US Army)
BAA96024 (Srivastava)
Heat Shock Protein-Peptide Complexes
As Antiviral Agents

Cancer Research Institute
Investigator Award

CAP CURE
Heat Shock Protein Based Prostate Cancer
Vaccine Starting from Single Cell or Small Biopsies

Teaching Experience:

Teach part of graduate immunology course (Tumor immunology) at Univ. of Connecticut.
Teach part of Advanced Immunology Course at University of Connecticut.
Taught complete graduate and undergraduate immunology courses at Fordham Univ., 1993 – 96.
Part of a graduate course in Biochemical Pharmacology (development of vaccines) (at Mount Sinai), 1988-93.
Part of an Advanced graduate immunology Course (tumor immunology) (Mount Sinai), 1992-93.
Medical students lectures on Cancer Chemotherapy and Cancer Immunology (Mount Sinai),1992-93.

Training Experience (graduate students, post-doctoral fellows etc.):

Thesis advisor to PhD students. Two MD PhD students Robert G Maki and Zihai Li, five straight PhD students: Daniel Levey, Nathalie Blachere, Sreyashi Basu, Robert Binder, Joseph Kovalchin and Ping Peng completed their PhD thesis in my laboratory between 1990 and now. Four MD/PhD students Marissa Caudill, Margaret Callahan, John Kelly and Ralph Vatner are presently working towards their dissertations.

Training Experience (graduate students, post-doctoral fellows etc.): (continued)

Five post-doctoral fellows (Sreyashi Basu, Robert Binder, Joseph Kovalchin, Toyoshi Matsutake and Ruibo Wang) presently work in my laboratory.
Twenty-two postdoctoral fellows (Anne Altmeyer, Kirstin Anderson, Rajiv Chandawarkar, Anna Feldweg, Oyvind Halaas, Michael Heike, Navdeep Jaikaria, Sylvia Janetzki, Stephanie Kespohl, Sumeet Kumar,

Kristi McQuade, Clyde Mendonca, Antoine Ménoret, Shin Oshima, Dirk Schadendorf, Ryuichiro Suto, Yasuaki Tamura, Heiichiro Udono, Mihir Wagh, Ruibo Wang, Siqing Wang and Gunner Weidt) have worked in my laboratory between 1988 and now.

Five of my students/trainees (Rajiv Chandawarkar, Michael Heike, Daniel Levey, Zihai Li, Antoine Ménoret, Dirk Schadendorf, and Heichiro Udono) have gone on to establish independent laboratories.

Two students, Michael Schneider (1992) and Steven McCoy (1993), worked towards a Westinghouse project and finished as semi-finalists.

A large number of medical students and Sigma Xi summer students rotate through my laboratory at any given time.

Administrative Experience:

At University of Connecticut School of Medicine:

Director, Center for Immunotherapy of Cancer and Infectious Diseases,	1/1/97- now
Deputy Director, University of Connecticut Cancer Center	10/1/03 - now
Director, University of Connecticut Cancer Center	1/1/01- 9/30/03
Member, Steering Committee, UConn General Clinical Research Ctr	12/1/98– 11/30/99

Various Search Committees

At Fordham University: Served as member of:
Institutional Animal Care and Use Committee
Radiation Safety Committee

At Mount Sinai School of Medicine: Served as member of:
Institutional Committee on Special Grants and Fellowships, 1990-1993;
Institutional Advisory Committee to Center for Laboratory Animal Sciences, 1989-93;
Cancer and Generic Lab Committees of Institute for Human Genomic Studies, 1992;
Departmental Graduate Teaching and Safety & Services Committees, 1988-1993;
Director of the Departmental seminar program, 1992-3.

Honors and Awards:

Merit Scholar, Government of India, 1969
Indian Council of Agricultural Research Award in Plant Physiology, 1973
Gold Medal from University of Allahbad, 1975
UNESCO International Studentship in Microbiology, 1978-1979
Senior Fellowship of Indian Council of Scientific and Industrial Research, 1980
John Hans and Edna Alice Old Postdoctoral Fellowship, Cancer Research Institute, NY 1984-1986
First Independent Research Support and Transition Award of NIH, 1987
Irma T. Hirshl Award, 1989

Honors and Awards :(continued)

Investigator Award of Cancer Research Institute, New York, 1989.
Mildred Scheel Lecturer at the International Conference on "Hyperthermia in Clinical Oncology", Munich, Germany, 1993
Listed in the International Directory of Distinguished Leadership, American Biographical Inst., 1994
Member, Experimental Immunology study section, NIH, 1994 - 1999.
Member, Scientific Advisory Council, Cancer Research Institute, New York, and Member, Fellowships Committee of the CRI, 1996 - present

Sigma Tau Foundation Lecturer, Rome, Italy, March 1997
UICC (Union Internationale Contre le cancer) Roll of Honor; inducted 1997
Who's Who in Medicine and Healthcare, 2000-2001
Founding Member, Academy of Cancer Immunology, New York
Klaus Irmscher Lecture, Wistar Institute, Philadelphia, PA, 2000

Membership in Societies :

American Association of Immunologists , American Association of Cancer Research International Interest Group in Biorecognition Technology, American Association for Advancement of Science, International Society for Vaccination (Charter member), Cell Stress Society (Life Member)

Publications: See Appendix 1

Patents: See Appendix 2

Organized Conferences: See Appendix 3

Invited Lectures and Talks: See Appendix 4

Personal information :

Born Sultanpur, U.P., India to Babu Mangla Prasad and Tara Devi Srivastava. Married to Jasmine Shah. One child Vasishth Vidyadhar, born 1991.

CORPORATE ACTIVITIES
(Companies founded and roles served in each)

ANTIGENICS

I am the scientific founder of Antigenics Inc., a for-profit company that is working to utilize the immunological activities of heat shock proteins for treatment of cancers, infections and autoimmune disorders. I founded the company in 1993-94 while I was Assistant Professor of Pharmacology at Mount Sinai School of Medicine. The company was founded on basis of the intellectual property and scientific

knowhow developed by my laboratory and with the business involvement of Garo Armen, presently the CEO of Antigenics. Antigenics is located in New York, NY and Lexington, MA. Antigenics was privately held till February 2001, at which time it went public and is since listed on NASDAQ (AGEN). I am a significant shareholder of Antigenics.

I have served on the Board of Directors of Antigenics since inception. I have also chaired the Scientific Advisory Board of Antigenics since the Company's inception. I am not and never have been an employee of the Company. I have been a full-time employee of Mount Sinai School of Medicine (till 1993), Fordham University (till 1996) and University of Connecticut (since 1997). I have always been a paid consultant to Antigenics and in that capacity, have been the chief architect of the Company's scientific, clinical, regulatory, manufacturing and business development activities. I have provided extensive support to the company in fund-raising at every round of financing.

For more details, please visit www.antigenics.com

IKONISYS

I became aware of the work of **Error! Reference source not found.** of the University of Connecticut and Dr. Phyllis Tafas of University of Athens, soon after my arrival at University of Connecticut. I helped Drs. Tsipouras and Tafas found a privately held company to utilize their work on rapid detection of rare cells in blood or other complex biological tissues. Ikionisys was founded in 1998 and is a privately held Company, with headquarters in Science park in New Haven, CT.

I served on the Board of Directors of Ikonisys from inception till early 2004. I am a significant shareholder of Ikonisys but do not have any executive or advisory roles in the Company.

For more details, please visit www.ikonisys.com

ASILAS

Operating under the auspices of a Switzerland-based private group, called CambriaTech (also founded by me and 4 additional partners), I helped found Asilas in order to utilize the expertise in mammalian genetics, of its scientific founders C.S.Shashikant (Penn State), Frank Ruddle (Yale) and Adrain Hayday (University of London). Asilas aims to create mouse models of human disease (with particular emphasis on neurodegenerative diseases) with a view to down stream partnering in drug development.

CORPORATE ACTIVITIES (continued)
(Companies founded and roles served in each)

ASILAS (continued)

Asilas is a very early stage company in the virtual stage. It does not have its own facilities at the moment. I am a significant shareholder in Asilas and am involved with it in an advisory/oversight capacity.

For more details, please visit www.asilas.com

MEDERGY

Operating under the auspices of a Switzerland-based private group, called CambriaTech (also founded by me and 4 additional partners), I helped found Medergy in order to utilize the expertise in mitochondrial

genetics, of its scientific founder Doug Wallace(University of California Irvine). Medergy is developing diagnostic reagents for and drugs against degenerative disorders of mitochondrial origin.

Asilas is a very early stage company in the virtual stage. It does not have its own facilities at the moment. I am a significant shareholder in Medergy and am involved with it in an advisory/oversight capacity.

For more details, please visit www.medergy.com

APPENDIX 1

Publications
(Abstracts are not included)

A. THESES:

Srivastava, P.K.: Gymnosperms of *Glossopleris* flora. M.Sc. Thesis, University of Allahabad, Allahabad, India, 1975

Srivastava, P.K. Cell surfaces during normal and abnormal growth: Purification of a tumor-associated antigen and a tumor-rejection antigen from a rat hepatoma. Ph.D. Thesis, Centre for Cellular and Molecular Biology, Osmania University, Hyderabad, India, 1983.

B. BOOKS:

Srivastava PK (ed.). Cellular Immunity to Cancer. ImmunoMethods Series, Academic Press, 1997.

Srivastava PK. (ed) Heat-Shock Protein-Immune System Interactions, Methods, Academic Press, 2003.

Srivastava PK Textbook of cancer immunology, John-Wiley, In preparation.

C. REVIEWS:

Das MR, Parnaik VK and Srivastava PK. Molecular biology of malignant transformation. Biochemical Reviews. 51: 47-60, 1981.

DeLeo AB and Srivastava PK. Cell surface antigens of chemically induced sarcomas of murine origin. Cancer Surveys. 41: 21-34, 1985.

Srivastava PK and Old LJ. Individually distinct transplantation antigens of chemically induced mouse tumors. Immunology Today. 9: 78-83, 1988. Also see Immunology Today, 10:78 for response to a letter.

Srivastava PK and Maki RG. Stress-Induced proteins as tumor antigens. Current Topics in Microbiology and Immunology 167 : 109 - 124, 1991.

Srivastava PK. Peptide - binding heat shock proteins in the endoplasmic reticulum: Role in immune response to cancer and in antigen presentation. Advances in Cancer Res. 62:153-177, 1993.

Srivastava PK and Udono H. Heat shock proteins in immune response to cancer: The Fourth Paradigm. Experientia 50(11-12): 1054-1060, 1994.

Blachere NE and Srivastava PK. Heat shock protein-based cancer vaccines and related thoughts on immunogenicity of human tumors. Seminars in Immunology 6 : 349-355, 1995.

Srivastava PK and Levey DL. Alterations in T cells of cancer-bearers: whence specificity? Immunology Today, 17 (8): 365-368, 1996.

C. REVIEWS: (continued)

Srivastava PK, Ménoret A, Basu S, Binder R, McQuade K. Heat shock proteins come of age: Primitive functions acquire new roles in an adaptive world. Immunity (8): 657-665, 1998.

Srivastava PK and Anderson K. Heat, heat shock, heat shock proteins and death: A central link in innate and adaptive immune responses, Immunology Letters , 74, 35-39, 2000.

Basu S and Srivastava PK. Heat shock proteins: the fountainhead of innate and adaptive immune responses. Cell Stress & Chaperones, 5, (5), 443-451, 2000.

Srivastava PK and Amato RJ. Heat shock proteins: The "Swiss Army Knife" Vaccines against cancers and infectious agents. *VACCINE*, 21;19(17-19):2590-7, 2001.

Srivastava PK. Interaction of Heat shock proteins with peptides and antigen presenting cells : Chaperoning of the innate and adaptive immune responses. Annual Review of Immunology, Vol. 20, 395-425, 2002.

Li Z and Srivastava PK. Heat Shock Proteins. Current Protocols in Immunology, In Press, 2003.

Srivastava PK. Heat shock proteins in innate and adaptive immunity. Nature Reviews Immunology, Vol. 2, 185-194, 2002.

Srivastava PK. Heat shock protein and immune response. Scientific American (under preparation upon invitation), 2004.

D. EDITORIALS and CRITICAL COMMENTARIES :

Srivastava, P.K. Protein tumor antigens. Current Opinion in Immunology 3 : 654 - 658, 1991.

Srivastava PK. Heat shock proteins in specific immunotherapy of cancer. Current Opinions in Immunology, 6(5): 728-732, 1994.

Janetzki S and Srivastava PK. Heat shock protein - peptide complexes as therapeutic vaccines against human cancer. Guest Editorial, Clinical Immunotherapeutics, 3: 325-329, 1995.

Srivastava PK. Do human cancers express shared protective antigens? or the necessity of remembrance of things past. Semin Immunol 8(5): 295-302., 1997.

Srivastava PK. Immunotherapy of human cancer: lessons from mice. Nature Immunology,1 (5), 363-366, 2000.

Li Z, Menoret A, Srivastava PK. Roles of heat-shock proteins in antigen presentation and cross presentation. Current Opinions in Immunology, 14(1), 45-51, 2002.

Srivastava PK. Heat Shock Proteins. Modern Aspects of Immunobiology, 2 (3), 104, 2002.

D. EDITORIALS and CRITICAL COMMENTARIES : (continued)

Stebbing J, Bower M, Srivastava P. Kaposi's sarcoma as a model for cancer immunotherapy. Trends in Molecular Medicine, In press, 2004.

Binder RJ, Vatner R, Srivastava PK. A critical contemplation of HSP receptors. Tissue Antigens, In press, 2004.

E. ORIGINAL PEER-REVIEWD PAPERS (laboratory research):

Srivastava,P.K., Harashima S. and Oshima, Y. Formation of two-spored asci interrupted sporulation in Saccharomyces cerevisiae. J. Gen. Microbiol. 123: 29-37, 1981.

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Srivastava PK, Kumar S, and Mendonca C. Principles and practice of the use of heat shock protein-peptide complexes for immunotherapy of human cancer. Biologic Therapy of Cancer Updates, Lippincott Williams & Wilkins, Vol. 2 No. 3, 2001.

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Because of pressures on time, I had to stop accepting invitations for book chapters since 2002, except under exceptional circumstances

APPENDIX 2

Patents

U.S. Patent no. 5,750,119: Immunotherapeutic stress protein-peptide complexes against cancer. Issued May 12, 1998

U.S. Patent no. 5,830,464: Compositions and methods for the treatment and growth inhibition of cancer using heat shock/stress protein-peptide complexes in combination with adoptive immunotherapy. Issued November 3,1998

U.S. Patent no. 5,837,251: Compositions and methods using complexes of heat shock proteins and antigenic molecules for the treatment and prevention of neoplastic diseases . Issued Nov. 3, 1998

U.S. Patent no. 5,935,576: Compositions and methods for the treatment and prevention of neoplastic diseases using heat shock proteins complexed with exogenous antigens. Issued August 10, 1999

U.S. Patent no. 5,948,646: Methods for preparation of vaccines against cancer comprising heat shock protein-peptide complexes. Issued September 7, 1999

U.S. Patent no. 5,961,979: Stress protein-peptide complexes as prophylactic and therapeutic vaccines against intracellular pathogens. Issued October 5, 1999

U.S. Patent no. 5,985,270: Adoptive immunotherapy using macrophages sensitized with heat shock protein-epitope complexes. Issued November 16,1999

U.S. Patent no. 5,997,873: Method of preparation of heat shock protein 70-peptide complexes. Issued December 7, 1999

U.S. Patent no. 6,007,821: Method and compositions for the treatment of autoimmune disease using heat shock proteins. Issued December 28,1999

U.S. Patent no. 6,017,540: Prevention and treatment of primary and metastatic neoplastic diseases and infectious dis. with heat shock/stress protein-peptide complexes. Issued Jan. 25, 2000

U.S. Patent no.6,017,544.Composition comprising immunogenic stress protein-peptide complexes against cancer and a cytokine. Issued January 25, 2000

U.S. Patent no.6,030,618:Therapeutic and prophylactic methods using heat shock proteins. Issued February 29, 2000

U.S. Patent no. 6,048,530: Stress protein-peptide complexes as prophylactic and therapeutic vaccines against intracellular pathogens. Issued April 11, 2000

U.S. Patent no.6,130,087:Methods for generating cytotoxic T cells in vitro.Issued October 10, 2000

U.S. Patent no. 6,136,315: Compositions and methods using complexes of heat shock protein 70 and antigenic molecules for the treatment and prevention of neoplastic diseases. Issued October 24, 2000

U.S. Patent no. 6,139,841: Compositions and methods using complexes of heat shock protein 70 and antigenic molecules for the treatment and prevention of infectious diseases. Issued October 31, 2000

U.S. Patent no. 6,143,299: Compositions and methods using complexes of heat shock protein gp96 and antigenic molecules for the treatment and prevention of infectious diseases. Issued November 7, 2000

U.S. Patent no. 6,156,302: Adoptive immunotherapy using macrophages sensitized with heat shock protein-epitope complexes. Issued December 5, 2000

U.S. Patent no. 6,162,436:Compositions and methods using complexes of heat shock protein 90 and

antigenic molecules for the treatment and prevention of neoplastic diseases. Issued December 19, 2000

U.S. Patent no. 6,168,793: Heat shock protein 70 preparations in vaccination against cancer and infectious disease. Issued January 2, 2001.

U.S. Patent no. 6,187,312: Compositions and methods using complexes of heat shock protein 90 and antigenic molecules for the treatment and prevention of infectious diseases. Issued: February 13, 2001

U.S. Patent no. 6,322,790: Compositions and methods for eliciting an immune response using heat shock/stress protein-peptide complexes in combination with adoptive immunotherapy. Issued: November 27, 2001

U.S. Patent no. 6,375,953: Treatment of infectious diseases with hsp70-peptide complexes. Issued: April 23, 2002

U.S. Patent no. 6,379,672: Prevention of infectious diseases with gp96-peptide complexes. Issued: April 30, 2002

U.S. Patent no. 6,383,491: Prevention of infectious diseases with hsp90-peptide complexes. Issued: May 7, 2002

U.S. Patent no. 6,383,492: Treatment of infectious diseases with gp96-peptide complexes. Issued: May 7, 2002

U.S. Patent no. 6,383,493: Methods and compositions for eliciting an immune response with hsp70-peptide complexes. Issued: May 7, 2002

U.S. Patent no. 6,383,494: Methods and compositions for eliciting an immune response with gp96-peptide complexes. Issued: May 7, 2002

U.S. Patent no. 6,387,374 Treatment of primary and metastatic neoplastic diseases with hsp90-peptide complexes. Issued May 14, 2002

U.S. Patent no. 6,391,306 Treatment of infectious diseases with hsp90-peptide complexes. Issued May 21, 2002

U.S. Patent no. 6,399,069 Prevention of infectious diseases with hsp70-peptide complexes. Issued June 4, 2002

U.S. Patent no. 6,399,070 Methods and compositions for eliciting an immune response with hsp90-peptide complexes. Issued June 4, 2002

U.S. Patent no. 6,403,095 Treatment of primary and metastatic neoplastic diseases with HSP70-peptide complexes. Issued June 11, 2002

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2002

U.S. Patent no. 6,410,026 Methods for preparation of vaccines against cancer. Issued June 25, 2002

U.S. Patent no. 6,410,027 Methods for preparation of vaccines against cancer. Issued June 25, 2002

U.S. Patent no. 6,410,028 Therapeutic and prophylactic methods using heat shock proteins. Issued June 25, 2002

U.S. Patent no. 6,436,404 Prevention of primary and metastatic neoplastic diseases with GP96-peptide complexes. Issued August 20, 2002

U.S. Patent no. 6,447,780 Prevention of primary and metastatic neoplastic diseases with hsp90-peptide complexes. Issued September 10, 2002

U.S. Patent no. 6,447,781 Therapeutic and prophylactic methods using heat shock proteins. Issued September 10, 2002

U.S. Patent no. 6,451,316 Methods for generating antigen-reactive T cells in vitro. Issued September 17, 2002

U.S. Patent no. 6,455,048 Prevention of primary and metastatic neoplastic diseases with hsp70-peptide complexes. Issued September 24, 2002

U.S. Patent no. 6,455,503 Stress protein-peptide complexes as prophylactic and therapeutic vaccines against intracellular pathogens. Issued September 24, 2002

U.S. Patent no. 6,461,615 Therapeutic and prophylactic methods using heat shock proteins. Issued October 8, 2002

U.S. Patent no. 6,468,540 Immunotherapeutic stress protein-peptide complexes against cancer. Issued October 22, 2002

U.S. Patent no. 6,475,490 Compositions and methods for promoting tissue repair using heat shock proteins. Issued November 5, 2002

In addition, over 50 applications are pending in the U.S. and foreign territories.

APPENDIX 3

Major Conferences Organized
(not including sessions within conferences)

I International Conference on Heat Shock Proteins in Immune Response, October 12-15, 1998, Farmington, CT

II International Conference on Heat Shock Proteins in Immune Response, October 8-12, 2000, Farmington, CT

Winter School in Immunology, Kovalam, Kerala, India, February 5-9, 2001, Organized by Cancer Research Institute, New York

III International Conference on Heat Shock Proteins in Immune Response, October 6-9, 2002, Farmington, CT

ll Winter School in Immunology, Kovalam, Kerala, India, December 16-20, 2002 Organized by Cancer Research Institute, New York

IV International Conference on Heat Shock Proteins in Immune Response, October 10-13, 2004, Farmington, CT

lll Winter School in Immunology, Kovalam, Kerala, India, December 16-20, 2003 Organized by Cancer Research Institute, New York

IV Winter School in Immunology, Kovalam, Kerala, India, December 16-20, 2004 Organized by Cancer Research Institute, New York

APPENDIX 4

Invited Lectures (selected list)

Since Fall of 2002, Dr. Srivastava has declined most invitations to give talks because of medical studies.

4th Annual Stem Cells & Regenerative Medicine, Princeton, NJ, October 18-19, 2004
Cancer Vaccines 2004 - The Next Decade, New York City, NY, October 4-6, 2004
1st International Conference on Basis & Clinical Immunogenomics, Budapest, Hungary, October 3-7, 2004
4th World Congress on Vaccines & Immunization, Japan, September 30-October 3, 2004
12th International Congress of Immunology (ICI) & 4th Annual Conference of Focis Montréal, Québec, Canada, July 18-23, 2004
ASCO Conference, 40th Annual Meeting, New Orleans, LA, June 4-7, 2004
Biochemical Society, Heat Shock Proteins & Modulation of Cellular Function, London, April 16, 2004
2nd International Conference, Strategies for Immune Therapy, Germany, February 29 - March 3, 2004
19th Annual Ella T. Grasso Memorial Conference, Farmington, CT, November 20, 2003
13th New England Regional Workshop on Autoimmune Diabetes, Woods Hole, MA, November 7, 2003
Johnson & Johnson Immune Modulation Symposium Malvern, Philadelphia, Pennsylvania, October 16, 2003
1st Annual European Conference: Perspectives in Melanoma Management, Amsterdam, The Netherlands October 9-11, 2003
CANCER VACCINES 2003-Cancer and HIV Vaccines: Shared Lessons, Manhatten, NY, October 1-3, 2003
First International Congress on Stress Responses in Biology and Medicine, Quebec City, Canada, September 10-14, 2003
International Summer School on Cancer Immunology and Immunotherapy, Ionian Village Peloponese, Greece, September 8 -13, 2003
First Annual Melanoma Research Congress, Philadelphia, PA, June 21-24, 2003
European Society of Hyperthermic Oncology ESHO2003, Munich, Germany, June 4-7, 2003
International Society for Cancer Gene Therapy Meeting, Singapore, 26th & 27th April 2003
ASCO Conference, 39th Annual Meeting, Chicago, IL, May 31-June 3, 2003
"CASC" Seminar - Rupert Sheldrake , Uconn, Farmington, CT, March 7, 2003
5th Annual Walker's Cay Collogquium on Cancer Vaccines and Immunotherapy, Abaco, Bahamas, March 5-8, 2003
Keystone Symposium, Basic Aspects of Tumor Immunology, February 17-23, 2003
American Association of Immunologists and NCI (NIH), "Research Opportunities in Cancer Immunology", Bethesda, MD, January 22-24, 2003
Winter School in Immunology, Kovalam, Kerala, India, December 16-20, 2002
BioSecurity, 2002, Las Vegas, MGM Grand, NV, November 18-21, 2002
New England Immunology Conference (NEIC), Woods Hole, MA, November 16-17, 2002
12th New England Regional Workshop on Autoimmune Diabetes Mellitus, Woods Hole, MA, November 15, 2002
UConn Immunology Graduate Retreat, Avon Old Farms Hotel, Avon, CT, November 15, 2002
Brazilian Society of Immunology (SBI), Salvador, Brazil, October 20-23, 2002
AACR Meeting on Cancer Prevention, New York City, NY, October 14-18, 2002
EMBO, "The biology of heat shock proteins and molecular chaperones", Warsaw, Poland, September 25 -29, 2002
HSP90 Workshop, Arolla, Swiss Alps, Switzerland, August 24-28, 2002
Upenn Immunology Training Grant Retreat, Philadelphia, PA, August 1, 2002
Eurocancer 2002, Paris France, June 4-6, 2002
ASCO, Orlando, FL May 17, 2002
Annual Conference on Vaccine Research, Baltimore, MD, May 6-8, 2002
DARPA 2002 UPC PI Conference, Lexington, KY, April 6-10, 2002
World Drug Discovery Summit, Copenhagen, Denmark, April 3-5, 2002
2002 Keystone Symposia, Keystone, Colorado, February 25-March 3, 2002
Australasian Society for Immunology Conference, Canberra, Australia, 2-5 December, 2001
III International Workshop on Molecular Biology of Stress Responses, Mendoza, Argentina, October 9-13, 2001
IV "Anton Dohrn" Workshop, "New Perspectives in Tunicate Biology", Ischia, Italy, September 29 – October 2, 2001
British Society of Histocompatibility and Immunogenetics, St John's College, Cambridge, London, September 26–28,2001

Invited Lectures (continued):

"CASC" Seminar, Uconn, Farmington, CT, September 10, 2001

8thCGGH Symposium "New Paradigms of Molecular Chaperones in the Postgenome Era", August 6-9, 2001, Sapporo Japan

11th International Congress of Immunology, "Molecular interactions in infection and immunity",Stockholm, Sweden, July 22-28, 2001

International Society of Cancer Gene Therapy, IVth Mtg., London, July 12-13, 2001

Perspectives in Melanoma V: Scientific and Clinical Foundation for Future Progress, The University of Pittsburgh, June 7-8, 2001, Pittsburgh, PA

Annual meeting of the "Hinterzartener Kreis" for Cancer Research, Cadenabbia/Como, Italy, May 10-13, 2001

7th National Symposium: Basic Aspects of Vaccines, Baltimore, MD, May 2-4, 2001

Winter School in Immunology, Kovalam, Kerala, India, February 8-13,2001

Keystone Symposia on Molecular and Cellular Biology, "Interfaces between innate and adaptive immunity, Keystone, CO, January 22-27, 2001

The British Society for Immunology Congress 2000, "Heat shock proteins: The fountainhead of innate and adaptive immune responses", London, December 5-8, 2000

University of London, Guy's King's & St. Thomas' Medical School, Annual Immunobiology Research Day, London, December 4, 2000

Dana Farber Cancer Institute, "International Symposium on Heat Shock Proteins in Biology and Medicine, Woods Hole, MA, November 6-8, 2000

II International Conf. on Heat Shock Proteins in Immune Response, Farmington, CT, October 8-12, 2000

Cancer Research Institute, Cancer Vaccines 2000, New York City, NY, October 2-4, 2000

EFIS 2000, European Federation of Immunological Societies, 14th European Immunology Meeting, Poznan, Poland, September 23-27, 2000

EFIS 2000, Heat Shock Proteins: Immune, Stress response and apoptosis, Gdansk, Poland, September 20-22, 2000

4th EFIS Tatra Immunology Conference, "Molecular Determinants of T Cell Immunity", Tatra Mountains, Slovakia, September 2-7, 2000

Proceedings of Millennium Second World Congress on Vaccines and Immunization, Liege, Belgium, August 29-September 2, 2000

University of CT Cancer Symposium, University of CT, Farmington, CT, May 11, 2000

DARPA 2000 BWD UPC/AD Conference, Fort Lauderdale, FL, January 30, 2000 – February 1, 2000

Annual Meeting of the Japanese Society for Immunology, Nagasaki University, Japan, November 28 – December 4, 1999

1st Annual Pathogenesis Symposium, University of CT, Farmington, CT, November 4, 1999

Drug Discovery for 21st Century, Worcester, MA, October 28, 1999

Cancer Research Institute, "Cancer Immunosurveillance", New York City, NY, October 4-6, 1999

1999 Annual Meeting, New England Surgical Society, Newport, RI, September 24-26, 1999

NIH – EI Study Section, Washington DC, June 17-18, 1999

Symposium of the SFB 432, "Immunological Mechanisms of Tumor Defense", Johannes Gutenberg-Universitat Mainz Klinikum, Mainz, Germany, February 26-27, 1999

Clinical Evaluation of 2nd Generation Cancer Vaccines, London, UK, February 24-25, 1999

DARPA Conference for Unconventional Pathogen Countermeasures, Monterey, CA, February 8-12, 1999

NMHCC Bio/Technology Division's Conference, Washington, DC, November 19-20, 1998

Robert Steel Foundation International Symposium 1998, Memorial Sloan-Kettering, "New Strategies for Stimulating and Augmenting Host Resistance to Malignant Cells", New York, NY, October 21-23, 1998

Cancer Research Institute Symposium, Cancer Vaccine Week 1998, New York City, New York, October 5-9,1998

AACR "Cellular Targets of Viral Carcinogenesis", Dana Point, California, September 24-28, 1998

National Cancer Institute, "Mechanisms of Immune Evasion by Tumors", Washington, DC, September 1-2, 1998

American Cancer Society 13th Annual Excalibur Round Table, Greenwich, CT August 13-14, 1998 Pfizer Mini-Symposium, "Novel Methods for Enhancement of Immune Responses to Peptides/Proteins", Groton, CT, July 24, 1998

2nd International Cancer Immunotherapy and Gene Therapy Conference, NMHCC Bio/Technology Conference Division, Waltham, Massachusetts, CT, June 15-16, 1998

Molecular Chaperones in Biology & Medicine, Mosbach, Germany, April 2-4, 1998

Symposium on Graft-versus-Host & Graft-versus-Leukemia Reactions 1998, Munich, Germany, March 28, 1998

Invited Lectures (continued):

Miami Nature Biotechnology Winter Symposia, Miami, FL, February 7-11, 1998

Immunological Attacks on Cancer, Cold Spring Harbor Laboratory, NY, October 19-22, 1997
International Workshop on Molecular Biology of Stress Response, Benares Hindu University, Varanasi, India, October 12-17, 1997
1997 Fourth Annual CapCURE Scientific Retreat, Lake Tahoe, Nevada, September 4-7, 1997
Molecular Virology and Vaccinology meeting of Drug Information Association, April 9-12, 1997, Newport Beach, California
UCLA symposium on "Cancer Immunity and Immunotherapy", Copper Mountain, Colorado, February 1997
Annual Meeting, American Society for Histocompatibility and Immunogenetics, San Diego, California, December 1996
Speaker and Organizing Committee member, Cancer Vaccines 1996, Cancer Research Institute, New York, October 1996
Plenary Speaker, Annual Meeting of German Society of Immunology, Hamburg, Germany, September 1996
European Immunology (ENII) meeting, Les Embiez, France, May 1996
Cancer Vaccines Conference (Henry Stewart Conference Studies), London, England, May 1996
Symposium in Immunology VI, Tumor Immunology, Organized by Immuno Pharma, Prague, Czechoslovakia, March 1996
European Heat Shock Meeting, Berlin, Germany, February-March 1996
Plenary Speaker, Annual Meeting of Canadian Society of Immunology, Montreal, Canada, March 1996
Plenary speaker, Annual Meeting of British Society of Histocompatibility and Immunogenetics, Liverpool, England, February 1996
Symposium speaker on cancer Vaccines, Annual Meeting, American Association of Advancement of Sciences, Baltimore, MD, February 1996
First International Workshop on Antigen Presentation, Oxnard, California, November 1995
Nobel Forum conference on Cancer Immunity, Stockholm, Sweden, September 21-23, 1995
Symposium speaker, IX International Congress of Immunology, 1995, San Francisco, July 1995
II International Conference on Engineered Vaccines against Cancer and AIDS, San Francisco, March 3-5, 1995
Deutsches Forschungsgemeinschaft, DFG workshop on Immunological aspects of heat shock proteins and heat shock response, December 1994
International Conference on "Cancer Vaccines", Cancer Research Inst., New York, October, 1994.
Distinguished Lecturer, University of Alabama, September 1994
IBC Conference on "Therapeutic Opportunities for Heat Shock Proteins", Cambridge, MA; September 29-30,1994
Johns Hopkins Oncology Center course on "New Approaches to Cancer Therapy", Baltimore, May 16, 1994
AACR Annual meeting, Symposium on "New Approaches to Cancer Immunotherapy", San Francisco, April 1994
Cold Spring Harbor Meeting on the 'Biology of heat shock proteins and molecular chaperones', May 4-8, 1994
German Society for Cell Biology meeting, Lbeck, Germany, March 1994
US - Japan Bionational Immunology Symposium, Bethesda, MD January 1994
Mildred Scheel Lecturer in the conference "Hyperthermia in Clinical Oncology", Ludwig Maximilians, University of Munich, Germany, November 1993
FASEB Conference on Tumor Immunotherapy, Vermont, June 9, 1993,
Workshop on "Heat shock in Multiple Sclerosis and other disorders", Galicia, Spain, MS Society, April 2-5, 1993
UCLA symposium on Cellular Immunity to Cancer, Taos, New Mexico, March 1993
American Association of Immunologists meeting, Anaheim, CA, Session on Tumor Antigens, April 1992
Plenary speaker, Annual Meeting of Austrian Society for Allergology and Immunology, Graz, May 1991
Chairman, Symposium on "Nature of Tumor Antigens" at the American Association of Immunologists meeting, New Orleans, Lousiana, June 1990
Special AACR symposium on 'Molecular Basis of Tumor Immunology', Virginia, May 20-22 1990
UCLA Symposium on 'T Cell Immunity to Cancers' January - February 1990
VII Int'l Congress of Immunology, Berlin, Workshop on Tumor Antigens, 1989
NIH workshop on 'Influence of MHC Expression on Tumor Growth' Annapolis, Maryland, 1988
UCLA Symposium on 'Human Tumor Antigens and Specific Tumor Therapy', 1988
Cancer Research Institute workshop on 'Recent Advances in Human Melanoma Research'; New York, 1987

Also gave invited talks at:

Duke University, Emory University, New York University, Cornell University Medical School, Johns Hopkins University, German Cancer Research Center (Deutsches Krebsforschungszentrum), Heidelberg, Yale University, Sloan-Kettering Institute, University

of Mainz (Germany), Max - Planck Institut at Freiburg, Germany, Ludwig Institute for Cancer Research, Stockholm, Sweden, Netherlands Cancer Institute, Amsterdam, Holland, Center for Cellular and Molecular Biology, Hyderabad, India, Albert Einstein School of Medicine, New York, National Cancer Institute, NIH, The Jackson laboratory, Bar Harbor, Chiron Corporation, University of Texas, Houston, TX., Washington University, St. Louis, MO., MD Anderson Cancer Center, Houston, TX., Boehringer-Manheim, Penzburg, Germany, University of Connecticut, Farmington, Sigma-Tau Company, Rome, Italy, University of Lund, Sweden; Stanford University, Palo Alto, University of California at Berkley, University of Pennsylvania, Pennsylvania State University, Oxford University, England; Michigan Cancer Foundation, Detroit, Michigan; Mayo Clinic, Rochester, MN; University of Southern California; University of California at
Norris; University of Tennessee, Cornell University, New York; Massachusetts General, Harvard Medical School, New York; Karmanos Cancer Institute, Detroit, Michigan; Boehringer Ingelheim, Ridgefield, Connecticut; University of
Rochester, Rochester, New York; Roswell Park Cancer Inst. , Buffalo,NY; Entremed Inc. ,Rockville, Maryland; Elan Pharmaceuicals, South San Francisco, California; University of Connecticut, Storrs, Connecticut; Stazione Zoologica Anton Dohrn, Naples, Italy; Fox Chase Cancer Ctr, Pennsylvania; Cleveland Ohio State Univ., Ohio; UCSF Cancer Ctr, San Francisco, California; Rockefeller University Hospital, New York City, NY; Wistar Institute, Philadelphia, PA; Case Western, Cleveland Ohio; University of Leeds, London; NCBS, Bombay, India; Baylor University, Houston, TX; Indian Institute of Science Bangalore, India; University of CT, CASC Series, Farmington, CT; University of Michigan, Ann Arbor, MI; Sequella Foundation, Rockville, MD; Johns Hopkins Univ, Baltimore MD; PTC Therapeutics, Plainfield, NJ; UMDNJ-New Jersey Medical Center, NJ; Medical College of Wisconsin, Milwaukee; University of Pittsburgh Cancer Institute, PA; Indiana University School of Medicine, Indianapolis, IN; Scripps Research Institute, La Jolla, CA; University of Toronto, Ontario, Canada; Weill Cornell Medical Ctr, NY;

EXHIBIT E

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(54) Title: IMMUNOMODULATORY PROPERTIES OF BIP

(57) Abstract: The present invention relates to substances having immunomodulatory properties and to the use of such substances for the treatment or prevention of an unwanted immune response. In particular, the present invention relates to the use of BiP or a functional fragment of homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, in the treatment or prevention of an unwanted immune response.

IMMUNOMODULATORY PROPERTIES OF BIP

The present invention relates to substances having immunomodulatory properties and to the uses of such substances for the treatment or prevention of an unwanted immune response. The present invention also relates to a pharmaceutical composition comprising a substance having immunomodulatory properties.

There are many situations in which unwanted immune responses lead directly to disease or interfere with therapy. Autoimmune diseases are one class of diseases of the first kind. Examples of the second kind of situation are responses which interfere either with the function of transplanted tissues and organs (allo- or xeno-graft rejection) or which inactivate substances used for gene therapy and which have not previously been encountered by the immune system of the recipient. A similar example of the latter kind is the response following the infusion of therapeutic proteins collectively known as 'biologics' of human or non-human origin, including monoclonal antibodies and other therapeutic proteins such as blood clotting factors, and enzymes. These varied situations are at present poorly managed and each demands separate therapeutic approaches.

The therapeutic approach of the present invention is based on BiP, the 78kD endoplasmic reticulum chaperone. In International patent application PCT/GB99/03316 (publication No. WO 00/21995), it has been demonstrated that recombinant BiP, expressed and purified from transfected *E. coli* was able to prevent the induction of collagen-induced arthritis (CIA) in susceptible DBA/1 mice. The isolation of BiP from human cells and cell lines has been fully described in WO 00/21995 as well as the cloning and expression of the DNA encoding this protein. The skilled person is therefore referred to WO 00/21995 for all necessary information relevant to the present application. WO 00/21955 is thus incorporated herein by reference. The BiP protein from human cells has a high degree of homology with BiP from other species and the term BiP is therefore used herein to embrace all such proteins which have the property of inducing IL-10. Minor variations on the specific

DNA and amino acid sequences disclosed in WO 00/21995 are also to be included provided the above property is retained as discussed further herein.

The inventors have researched the mode of action of the BiP protein and have confirmed that it has a general immunomodulatory property which is of application to the treatment of diseases other than rheumatoid arthritis. In particular, the inventors have found that BiP can be used in preventing adjuvant arthritis (AA) in Lewis rats. As can be seen from the experiments described below, BiP was able to significantly inhibit the development of AA.

The present invention provides the use of BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, in the manufacture of a medicament for the treatment or prevention of an unwanted immune response.

It is preferred that the use according to the present invention is not for the treatment or prevention of rheumatoid arthritis (RA) or collagen-induced arthritis (CIA).

It is further preferred that the use of the present invention is for preventing an unwanted immune response.

The term "BiP" as used herein refers to the 78kD endoplasmic reticulum chaperone protein as defined in WO 00/21995. It has been found that BiP causes: CD14+ cells to release IL-10; stimulates CD8+ cells to proliferate and release IL-10; inhibits the recall antigen response; and activates the expression of an array of anti-inflammatory genes in monocytes, including the migration inhibitory factor (MIF), the soluble TNF receptor II and TIMPs. Preferably, the BiP protein has the amino acid sequence given in WO 00/21995 as SEQ ID NO. 1 or SEQ ID NO. 2.

The term "a functional fragment" as used herein refers to a fragment of BiP which is capable of eliciting at least part of an activity of the full BiP protein. In particular, it is preferred that the functional fragment has at least one of the following functions: causes

CD14+ cells to release IL-10; stimulates CD8+ cells to proliferate and release IL-10; inhibits the recall antigen response; or activates the expression of an array of anti-inflammatory genes in monocytes, including the migration inhibitory factor (MIF), the soluble TNF receptor II and TIMPs. Preferably the functional fragment is at least 20 amino acids, more preferably at least 50 amino acids and most preferably at least 100 amino acids in length. Particularly preferred fragments comprise a conserved region which has been found to be homologous to a number of naturally occurring BiP proteins. Such conserved regions are considered to have a specific function.

The term "a functional homolog" as used herein refers to a homolog that retains at least part of an activity of the BiP protein described in WO 00/21995. In particular, it is preferred that the functional homolog has at least one of the following functions: causes CD14+ cells to release IL-10; stimulates CD8+ cells to proliferate and release IL-10; inhibits the recall antigen response; or activates the expression of an array of anti-inflammatory genes in monocytes, including the migration inhibitory factor (MIF), the soluble TNF receptor II and TIMPs. It is preferred that the functional homolog has at least 80%, more preferably at least 90% and most preferably at least 95% amino acid sequence homology with one of the BiP proteins described in WO 00/21995. Preferably the sequence homology is measured by using BLAST analysis. It is particularly preferred that the functional homolog differs by only 1 to 20 amino acids from one of the BiP proteins described in WO 00/21995. It is further preferred that the amino acid changes are conservative. Conservative changes are those that replace one amino acid with one from the family of amino acids which are related in their side chains. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar conservative replacement of an amino acid with a structurally related amino acid will not have a major effect on the biological activity of the protein. Mutations which increase the number of amino acids which are capable of forming disulphide bonds with other amino acids in the protein are particularly preferred in order to increase the stability of the protein.

The nucleic acid molecule used in the present invention encodes BiP or a functional fragment or homolog thereof as defined above. The nucleic acid molecule can be obtained by methods well known in the art. For example, naturally occurring sequences may be obtained by genomic cloning or cDNA cloning from suitable cell lines or from DNA or cDNA derived directly from the tissues of an organism, such as a human or mouse. Positive clones may be screened using appropriate probes for the nucleotide molecule desired. PCR cloning may also be used. The probes and primers can be easily generated given that the sequence of BiP is known (see WO 00/21995). Preferably the nucleic acid molecule has the sequence given in WO 00/21995 as SEQ ID NO. 3.

Numerous standard techniques known in the field of molecular biology may be used to prepare the desired nucleic acid or the probes and primers for identifying the positive clones. The nucleotide molecules probes or primers may be synthesised completely using standard oligonucleotide synthesis methods, such as the phosphoramidite method.

Numerous techniques may be used to alter the nucleic acid sequence obtained by the synthesis or cloning procedures, and such techniques are well known to those skilled in the art. For example, site directed mutagenesis, oligonucleotide directed mutagenesis and PCR techniques may be used to alter the DNA sequence. Such techniques are well known to those skilled in the art and are described in the vast body of literature known to those skilled in the art, for example Sambrook *et al.*, (1989).

The nucleic acid is preferably in the form of a vector comprising the necessary elements leading to the expression of the nucleic acid sequence encoding BiP or a functional fragment or homolog thereof. For example, it is preferred that the vector comprises a promoter operably linked to the nucleic acid sequence and a transcription termination sequence. Suitable promoters, transcription termination sequences and other functional elements required to obtain expression of the nucleic acid are well known to those skilled in the art.

The nucleic acid may be delivered to the individual using any method. For example, the nucleic acid may be delivered as a free nucleic acid, in the form of a viral delivery vector such as an adenovirus, contained in a liposome or via any known method.

The unwanted immune response may be any unwanted immune response. Specific unwanted immune responses are discussed in details below.

A Prevention of unwanted immune responses

i) Prevention of rejection of allo- and xeno- transplants

The rejection of allo- and xeno- transplants (TX) is a major problem barrier in the more effective use of TX for the therapy of organ failure. Present anti-rejection regimens are expensive, require life-long administration, may produce toxic side effects and are not universally effective. TX are grafted at a precisely known time. Just as the inventors have shown that BiP is able to prevent CIA and AA if given, respectively, at the time of or before the induction of arthritis, it is recommended to administer BiP just before or at the time of TX to prevent rejection. TX that may be beneficially treated in this way include all TX of tissues and organs, whether solid (for example, liver, kidney) or single cell (for example, blood cells, bone marrow cells or stem cells).

ii) Prevention of immune response to biologic therapeutic substances

A range of biologic therapies are used in clinical medicine. These include products from non-human sources and products from human sources. The biologics may be purified from a natural source, produced by recombinant gene technology, secreted after transfection of genes, or synthesised. These biologics may be proteins, glycoproteins or complex sugars. A disadvantage is that this has the potential to induce an immune response when administered to an immunologically naïve individual.

There are two main consequences of the induction of such an immune response. The first is the development of anaphylactic shock that may be life threatening. The second is the loss of therapeutic activity of the product because of the development of neutralising antibodies during the course of the immune response. It is recommended to administer BiP before or at the time of these interventions in order to prevent the

development of this unwanted immune response and thus prevent these therapeutic failures.

B Treatment of existing diseases

Existing immune-mediated diseases such as type I diabetes mellitus (in the early phase before complete destruction of the beta cells in the islets of Langerhans), thyroiditis, multiple sclerosis and diseases in which the immune system is activated are also open to treatment by the parenteral administration of BiP. This is because BiP releases IL-10 and other regulatory molecules from target cells such as CD14+ monocytes and CD8+ T cells.

According to a first embodiment of the use of the present invention the unwanted immune response is associated with an immune-mediated disease. Immune-mediated diseases include auto-immune diseases. Specific immune-mediated diseases include type-1 diabetes, thyroididtis, multiple sclerosis, systemic lupus erythematosus, Crohn's disease and all forms of viral and autoimmune hepatitis.

It is preferred that the use according the first embodiment of the present invention additionally comprises the use of an agent for enhancing the treatment or prevention of the immune-mediated disease in the manufacture of the medicament.

The agent for enhancing the treatment or prevention of the immune-mediated disease may be any agent including IL-10, IL-4, IL-11, TGF-beta, IL-13 and soluble cytokine receptors such as IL-1Ra, IL-1 and TNF soluble receptors.

Preferably the medicament is for administration to an individual suffering from or susceptible to developing an immune-mediated disease. Methods are know for determining whether an individual is suffering from an immune-mediated disease and methods are known for determining if an individual is likely to develop an immune-mediated disease. Methods for determining whether an individual is likely to

develop an immune include analysing risk factors such as genetic markers or environmental influences such as diet, etc.

In use, the medicament obtained by the use according to the first embodiment of the present invention is preferably administered to an individual before the immune-mediated disease develops or as soon as the immune-mediated disease has been diagnosed.

According to a second embodiment of the use of the present invention the unwanted immune response is associated with the rejection of a transplanted organ, tissue or cells.

The rejection response is well know and occurs when donated tissue is recognised as foreign by the recipient's immune system. The rejection response occurs with transplanted organs, such as heart, lung, kidney, liver, etc., transplanted tissues, such as skin, muscle tissue, etc., and with transplanted cells, such as bone marrow cells and stem cells.

It is preferred that the use according the second embodiment of the present invention additionally comprises the use of an agent for enhancing the treatment or prevention of the immune response associated with rejection of a transplanted organ, tissue or cells, in the manufacture of the medicament.

The agent for enhancing the treatment or prevention of the immune response associated with the rejection of transplanted organs, tissue or cells may be any agent that suppresses the immune system including glucorticoids, cyclosporin A, azathioprine, rapamycin and tacrolimus.

In use, the medicament obtained by the use according to the second embodiment of the present invention is preferably administered to an individual before or at substantially the same time as the transplantation of the organ, tissue or cells.

According to a third embodiment of the use of the present invention the unwanted immune response is the immune response to a biologic.

A biologic is any therapeutic agent given to an individual. The biologic may be from non-human or human sources. The biolgic may be a protein molecule (i.e. an enzyme, an antibody molecule, receptor ligand, etc), a glycoprotein, a polypeptide, peptide, carbohydrate, or an organic or inorganic chemical compound.

The use of biologics can cause unwanted immune responses. For example, an immune response can be raised against the biologic which may prevent the therapeutic activity of the biologic. Alternatively, the immune response may be so large that it lead to anaphylatic shock. For example, anti-TNFα therapy has resulted in the shortening of the interval between dosing (infliximab) this increasing the cost, and its use has been limited by anaphylaxis.

It is preferred that the use according the third embodiment of the present invention additionally comprises the use of an agent for enhancing the treatment or prevention of the immune response to the biologic.

The agent for enhancing the treatment or prevention of the immune response to the biologic may be any agent that suppresses the immune system including glucorticoids, cyclosporin A, azathioprine, rapamycin and tacrolimus.

In use, the medicament obtained by the use according to the third embodiment of the present invention is preferably administered to an individual before or at substantially the same time as the biologic.

The present invention also provides the use of BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, for stimulating the release of IL-10 from cells capable of releasing IL-10. Preferably the cells are peripheral blood mononuclear cells (PBMCs). Preferably, the PMBCs are CD14+ monocytes and/or CD8+ T cells and/or CD4+ T cells.

Preferably BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, is used to stimulate the release of IL-10 from PBMCs *in vitro* or *ex vivo*.

Preferably BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof is used to additionally stimulates gene expression of at least one of monocyte migration inhibitory factor (MIP), soluble TNF receptor II, IL-10 anti-inflammatory mediators and tissue inhibitor of matrix metalloproteinases (TIMP). Preferably BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, does not stimulate gene expression of matrix metalloproteinases (MMPs), monocyte chemoattractant protein (MCP-1) or TNFα.

The present invention also provides a pharmaceutical preparation comprising BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, in combination with a pharmaceutically acceptable carrier for use in the treatment or prevention of an unwanted immune response.

Preferably the pharmaceutical composition additionally comprises an agent for enhancing the treatment or prevention of the unwanted immune response.

The pharmaceutical composition of the present invention comprises a therapeutically effective amount of BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat or prevent the unwanted immune response.

For any agent, the therapeutically effective dose can be estimated initially either in cell culture assays, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise effective amount for a human subject will depend upon the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, an effective dose will be from 0.01 mg/kg to 50 mg/kg, preferably 0.05 mg/kg to 10 mg/kg.

Pharmaceutical compositions of this invention comprise BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, with any pharmaceutically acceptable carrier, adjuvant or vehicle. Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene- polyoxypropylene-block polymers, polyethylene glycol and wool fat.

The pharmaceutical compositions of this invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. Oral administration or administration by injection are preferred. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or

wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant such as Ph. Helv or a similar alcohol.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, and aqueous suspensions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The pharmaceutical compositions of this invention may also be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a novel agent of this invention with a suitable non- irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

Topical administration of the pharmaceutical compositions of this invention is especially useful when the desired treatment involves areas or organs readily accessible by topical application. For application topically to the skin, the pharmaceutical

composition should be formulated with a suitable ointment containing the active components suspended or dissolved in a carrier. Carriers for topical administration of the novel agents of this invention include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical composition can be formulated with a suitable lotion or cream containing the active agent suspended or dissolved in a carrier. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. The pharmaceutical compositions of this invention may also be topically applied to the lower intestinal tract by rectal suppository formulation or in a suitable enema formulation. Topically-transdermal patches are also included in this invention.

The pharmaceutical compositions of this invention may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

The present invention also provides a method of treating or preventing an unwanted immune response comprising administrating to an individual in need of such treatment an effective dose of the pharmaceutical composition according to the present invention.

As indicated above, there are many routes of administration of BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, including intravenous, intramuscular, nasal, oral, cutaneous, and topical. In particular, details of several preferred approaches to using BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof are described below.

(a) <u>Induction of mucosal tolerance</u>.

Delivery of BiP (p78) autoantigen or functional peptides derived therefrom by mucosal routes, e.g. through the intestine or nasal mucosa, alters the immune response by downregulating disease activity leaving the patient's immune system otherwise intact. Alternatively p78 or functional p78 peptides can be delivered as a nucleic acid molecule encoding them within an appropriate mammalian expression vector.

(b) Vaccination with TCR peptides

Peptides of the CDR3 region of the T cell receptor Valpha and Vbeta chains can be synthesised and used as vaccines for delivery by intradermal or intramuscular injection (see Kotzin *et al.*, Arthritis Rheum., 11, 1906-1919, 1998). BiP or a functional fragment or homolog thereof can be used in the same way.

(c) MHC blockade with native or altered peptides

BiP or a functional fragment or homolog thereof, may be given parenterally or orally in appropriate cases either unmodified or modified by amino acid substitution and/or attachment of chemical groupings so as to block MHC and especially HLA-DR4 thereby leading to suppression of T cell activation and disease. BiP or a functional fragment or homolog thereof, may be combined with soluble HLA-DR4 molecules and applied parenterally or orally.

(d) Induction of tolerance by plasmid DNA immunisation

Plasmids consisting of nucleic acid coding for BiP or a functional fragment or homolog thereof, may be given by injection. DNA coding for human IL-10, IL-4, IL-11, or TGF-beta, incorporated singly or in any combination, may be used to deviate the immune response to BiP towards a TH2 mode so as to suppress disease.

The present invention is now described by way of example only with reference to the following Figures:

Figure 1 shows the prevention of adjuvant arthritis by BiP. Male Lewis rats (n=5) were immunised with 50 μg PBS/DDA in each hind footpad (ie 100 μg/rat). Control animals (n=5) received only the PBS/DDA mixture. Thirteen days later, adjuvant arthritis was induced by a single intradermal injection of 0.5 mg *M.tuberculosis* in 100 μl IFA in the base of the tail.

Figure 2 shows the results of the investigation into BiP binding to peripheral blood mononuclear cell (PBMC) populations and fibroblast like synoviocytes by double immunofluorescence. Column A shows PBMC stained with human serum albumin (HSA) fluorescein isothiocyanate (FITC), as the negative control, and column B, FITC conjugated BiP, the PBMC were double stained with CD14, CD20, CD4, CD8, CD56. Column C shows BiP.FITC binding to 2 rheumatoid arthritis fibroblast-like synoviocytes compared with the HSA.FITC negative control.

Figure 3 shows IL-10 production following culture of peripheral blood mononuclear cells (PBMC) with BiP (20 μg/ml), beta-galactosidase (b-gal) (20 μg/ml) or lipopolysaccharide (LPS) (20 ng/ml) in the absence (A) or presence (B) of polymixin B for 24 hours. Culture supernatants were collected and IL-10 was measured by ELISA.

Figure 4 shows the proliferation of CD8 clone FC2B5 to BiP (closed circles) and control antigen β-galactosidase (open circles). This clone was generated from the peripheral blood of a normal individual. This profile is representative of other BiP responsive clones.

Figure 5 shows the cytokine profiles of BiP responsive clones and lines. The cytokine levels were measured in supernatants of cells previously shown to be BiP responsive, stimulated by mitogen. The profiles are compared with irradiated feeder cells alone (first data set).

Figure 6 shows the proliferative response of T cells to BiP stimulation.

Figure 7 shows BiP-driven T cell cytokine production from animals immunised with BiP.

Figure 8 shows BiP driven T cell cytokine production from control animals.

Figure 9 shows the uptake of tritiated thymidine following an allogeneic reaction between peripheral blood monocytes (MO) either cultured for 5 days in tissue culture medium, or matured with granulocyte macrophage-colony stimulating factor (GM-CSF) + IL-4, or with GM-CSF + IL-4 + BiP, prior to irradiation and culture with allogeneic peripheral blood mononuclear cells.

Figure 10 shows the proliferation response of peripheral blood mononuclear cells measured by the uptake of tritiated thymidine in either unstimulated cultures (TCM) or cultures stimulated with BiP (20μg/ml) or tuberculin PPD (10μg/ml) or with BiP and PPD.

EXAMPLES

MATERIALS and METHODS

Fluorescein isothiocyanate labelling of proteins

BiP or human serum albumin (HSA) were prepared at a concentration of 2 mg/ml in carbonate buffer 0.1M pH9.6. A stock solution of fluorescein isothiocyanate (FITC) was prepared at 10 mg/ml in carbonate buffer 0.1M pH 9.6. 50 μg FITC/mg protein was added to the protein solution in a glass container covered in foil. The solution was placed on a circular mixer and incubated at room temperature for 2 hours. The FITC labelled protein was then placed in dialysis tubing (which had been boiled for 5 mins with each of three changes of fresh distilled water) and dialysed overnight in 5 litres of phosphate buffered saline (PBS) (0.15M NaCl, 4mM NaH_2PO_4, 0.01M Na_2HPO_4 pH

7.2) followed by two further changes of 5 litres PBS. FITC labelled proteins were aliquoted and stored at 4°C.

Immunfluorescent staining of cells

PBMC were separated on Lymphoprep (Nycomed Amersham, Amersham, UK) by density centrifugation at 800g and then washed three times in Hanks buffered saline solution (Life Technologies, Paisley UK). Fibroblast-like synoviocytes or other adherent cells grown in culture were gently scraped from the surface of the flask before immunofluorescenct staining. The cells were pelleted and resuspended in PBS containing 0.5% bovine serum albumin and 0.01% azide (PBS/BSA/az). Cells at 10^5-10^6/ml were used for the staining. 100µl of cells were placed in a tube and 10µl of 1/5 normal human serum added. The cells are incubated on ice for 10 minutes and then washed twice at 300g in PBS/BSA/az at 4°C. The required amount of the FITC conjugated protein was then added to the cells in conjunction with any other protein directly conjugated to a different fluorochrome, such as phycoerythrin (PE), and the tube vortexed. The actual amount of protein added must be determined for each conjugation by a dose response curve. The cells were incubated on ice for 20 minutes and then washed twice at 300g in PBS/BSA/az at 4°C.

BiP.FITC was used at 1/50 dilution and counter-stained with anti-CD20 (B cell marker), CD3, CD4, CD8 (T cell markers), CD56 (NK cell marker), or CD14 (monocyte marker) all directly PE conjugated and used at 5µl/100µl (Becton Dickinson, Oxford, UK). After the final wash the cells were fixed in PBS/BSA/az with 1% paraformaldehyde in 250µl aliquots. The cells were then analysed on a FACScan using Cellquest software (Becton Dickinson, Oxford, UK).

Determination of cytokine production by PBMC stimulated with BiP

Supernatants from cultures PBMC (10^6/ml) either unstimulated or stimulated by BiP (20µg/ml) were harvested after 24 hours incubation. All cytokines were measured by commercial ELISA obtained from Pharmingen, Oxford, UK following 24h stimulation.

Cloning procedure for BiP responsive cells

Cloning of specific T cells:

Mononuclear cells were plated at $1x10^6$ cells ml^{-1} in 2ml culture wells in the presence of $20\mu gml^{-1}$ BiP (this concentration has been previously shown to be optimal for proliferation) in culture medium (RPMI 1640 + 10% human serum, L-glutamine and penicillin and streptomycin. Cells were cultured at 37^oC in 5% CO_2. After 7 days Lymphocult-T (LC-T) was added to the cultures ($40\mu lml^{-1}$) as a source of interleukin-2 (IL-2). After a further 7 days $1x10^6$ irradiated autologous feeder cells were added to each well with $40\mu lml^{-1}$ LC-T and $20\mu gml^{-1}$ BiP. This regime was continued for 3 rounds of feeder cells and then the cells plated at 10 cells per well into 96U plates with $1x10^4$ γ-irradiated allogeneic feeder cells (4000 Rads: ^{137}Cesium source) and $2\mu gml^{-1}$ Phytohaemagglutinin (PHA). After one week LC-T was added to the wells ($40\mu lml^{-1}$) and after a further week $1x10^4$ irradiated feeder cells, Lymphocult-T and PHA was added again. The cells were expanded into progressively bigger wells using this regime, until sufficient cell numbers were achieved for further study.

Proliferation assays.

When sufficient cell numbers were achieved $1x10^4$ cloned cells were incubated for three days with $1x10^5$ irradiated autologous feeder cells in the presence or absence of BiP ($20\mu gml^{-1}$) or PHA ($2\mu gml^{-1}$).

The cells were incubated for the last 18 hours with ^{3}H-thymidine ($0.2\mu Ci$) and then harvested. Proliferation was expressed as a stimulation index (SI): proliferation in the presence of stimulant/proliferation in the presence of medium alone.

Determination of clonality:

Phenotypic analysis was carried out on responding clones using CD3, 4 and 8 (BD). Briefly, cells were washed in FACS buffer (phosphate buffered saline containing 1% bovine serum albumin and 0.05% sodium azide) and incubated with 4µl of the appropriate antibodies. Three-colour analysis was performed using a FACScan flow cytometer and cell-quest software. T cell receptor usage was determined using a panel of

both FITC conjugated and non-conjugated antibodies. Briefly, for conjugated antibodies $1x10^4$ cells were incubated with 4µl of each of the FITC conjugated V beta specific antibodies. Clones were also stained with 4µl of both anti-CD4 conjugated to FITC and CD8 conjugated to PE. Where non-conjugated antibodies were used cells were incubated for 40 mins on ice with the primary antibody, washed twice in FACS buffer, then incubated for a further 40 mins with a FITC conjugated goat antibody raised against mouse immunoglobulins.

Stained cells were run on a FACScan flow cytometer with a 488nm laser and the results analysed using Cellquest and WinMDI analytical software.

Cytokine determination.

Supernatants were removed from cultures 24 hours after the last round of stimulation. Supernatants from cultures containing only irradiated feeders, LC-T and PHA were used as controls. The amount of interleukin (IL) 4 and IL-1, and γ-interferon and tumour necrosis factor-α were determined by ELISA (Pharmingen, according to manufacturers instructions). Briefly, plates (Nunc Maxisorp) were coated with a cytokine specific capture antibody, blocked with 10% foetal calf serum (FCS: to stop non-specific binding) and the supernatants incubated on the plate overnight at 4°C. Bound cytokine was detected with a biotin conjugated detection antibody and visualised with streptavidin conjugated horseradish peroxidase and TMB.

Inhibition of recall antigen responses by PBMC preincubated with BiP

PBMC were cultured either alone or with BiP (20µg/ml) for 0, 24, 48 or 72 hours prior to the addition of tuberculin purified protein derivative (PPD)(10µg/ml) and the cells incubated for 6 days following the addition of PPD. Tritiated thymidine was added to the cultures for the final 6 hours. The cells were then harvested and the uptake of tritiated thymidine assayed using a dry matrix beta counter (Canbarra-Packard, Pangbourne, UK).

Cytokine expression array:

Monocytes (MO) were separated by negative selection using an immunomagnetic bead kit (Dynal, Wirral, UK) and placed in culture at 2.10^6/ml for 24hours either alone or stimulated with BiP(20μg/ml) or with PMA (10ng/ml) +IONO(250ng/ml). The supernatants were harvested for ELISA and the cells were processed for extraction of total RNA and production of cDNA using oligo d(T) primers and reverse transcriptase. The expression array (R&D Systems, Oxford, UK) was used according to the manufacturer's instructions.

MECHANISM OF ACTION OF BIP IN PREVENTING AA AND CIA

A Stimulating release IL-10 from human CD14+ monocytes

The ability of BiP to prevent CIA and AA suggested to the inventors that it may have a generic ability to downregulate immune responses. In order to test this possibility, BiP was conjugated to fluorescein isothiyocyanate (FITC) and used in flow cytometry. As can be seen from Figure 2, BiP binds to different populations of cells found in human peripheral blood but especially to human CD14+ monocytes.

When peripheral human blood mononuclear cells were cultured with BiP, cells were stimulated to release interleukin 10 (IL-10) as well as tumour necrosis factor (TNF) α (see Figure 3). It is important to note that the amounts of secreted IL-10 are significantly greater than those for TNFα. Since IL-10 is anti-inflammatory and downmodulates immune responses while TNFα is pro-inflammatory, the administration of BiP will lead to the induction of an anti-inflammatory and downmodulatory immune environment thus explaining its ability to prevent the induction of CIA and AA.

B Stimulating release from CD8+ T cells

CD8+ cells from peripheral blood of humans subjects may be stimulated by BiP to proliferate (Figure 4). Indeed, the inventors have generated clones of such CD8+ T cells. These clones do not secrete the pro-inflammatory cytokine interferon (IFN)γ but do secrete IL-10 (Figure 5). Thus these cells have the characteristics of Tcl regulatory CD8+ T cells and are able to downmodulate immune responses.

C **Inhibition of recall antigen responses by PBMC pre-treated with BiP**

Pre-incubation of PBMC with BiP induced inhibition of the response to tuberculin purified protein derivative (PPD), a recall antigen. The inhibition increased with the length of time the cells had been exposed to BiP prior to the PPD challenge. (Inhibition of PPD responses: PBMC+BiP, 0h, 44.5 ± 30%, range 0-67%; 24h, 47.2 ± 35.8%, range 0-87%; 48h, 59.2 ± 27%, range 30-90%; 72h, 64 ± 23.6%, range 33-90%)

D **BiP induces monocytes to activate a more anti-inflammatory array of genes compared with activation by phorbol myristic acid (PMA) and calcium ionophore (IONO).**

An expression array (R&D Systems,Oxford, UK) capable of screening 375 different genes was used to analyse the gene activation profile of resting monocytes or that following BiP or PMA+IONO activation. The preliminary results show that those genes activated by BiP were more anti-inflammatory than those activated following direct cell activation by PMA+IONO. Differences are shown in Table 1. To be noted should be the following facts: BiP specifically induced gene activation for monocyte migration inhibitory factor (MIF), soluble TNF receptor II and IL-10 anti-inflammatory mediators, upregulated tissue inhibitor of matrix metalloproteinases (TIMP) but did not induce matrix metalloproteinase (MMP) or monocyte chemoattractant protein (MCP)-1, potent pathogenic mediators, or the inflammatory cytokine, TNFα. In contrast, PMA +IONO activation of monocytes upregulated many inflammatory mediators, such as TNF α, MCP-1 and MMP-1, 9 and-10.

Table 1

	Rest MO	BiP MO	BiP MO	PMA/IONO MO
Chemokines and receptors				
ENA-78	Y**	Y	Y	Y
MIP-1alpha		Y*	Y	Y
MIP-1beta		Y*	Y	
GRO alpha	Y	Y	Y	Y*
GRO beta	Y	Y	Y	Y*
GRO gamma	Y	Y	Y	Y*
IL-8	Y*	Y*	Y*	Y**
MCP-1				Y
MCP-3				Y
CCR1				Y
CXCR4			Y	
LDGF	Y	Y	Y	
PARC		Y		Y
Orphan receptors				
RDC-1		Y	Y	
ChemR23		Y	Y	Y
PARC		Y		Y
Proteinases or related factor				
Caspase		Y		Y
MMP-1				Y
MMP-9				Y
MMP-10				Y
TIMP-1	Y	Y	Y	Y
Urokinase R	Y	Y*	Y*	Y
Caspase 1		Y		Y
Integrins				
Integrin beta 1	Y	Y	Y	Y
Integrin beta-2	Y	Y	Y	Y
Integrin beta-4	Y		Y	Y
TGF superfamily				
Activin A	Y	Y	Y	Y*
TNF superfamily				
LIGHT	Y	Y	Y	Y
Cytokines and inhibitors				
IL-1beta	Y**	Y**	Y**	Y**
IL-1Ra	Y	Y	Y	Y
IL-6	Y	Y	Y	Y
IL-10		Y		
TNF alpha				Y
GM-CSF				Y
MIF		Y	Y	
Cytokine receptors				
IFNgamma RI	Y			
IFNgamma RII	Y			Y
IL-7R alpha	Y	Y	Y	
TNFRII		Y		
CD14	Y*	Y	Y	Y

Expression array of the genes activated in resting monocytes (REST MO), or stimulated by BiP(BiP MO), or by PMA+IONO (PMA+IONO MO). Y indicated the gene was activated and mRNA was present, * indicated a high level of expression. Only the positive genes have been included in the table.

***In vitro* functional studies with BiP**

The functional consequences of the addition of BiP has been investigated in two important experimental systems:

1) The allogeneic reaction is the *in vitro* equivalent of transplantation

2) Tuberculin purified protein derivative (PPD) stimulation is a measure of the lymphocytes response to recall antigens (an antigen to which the subject has already been immunised).

Figure 9 shows that allogeneic peripheral blood lymphocytes react to the resting monocytes with a low response. When the monocytes are matured into dendritic cells, using the well established technique of adding granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) for 5 days, the response by the allogeneic PBMC is greatly increased. When BiP is added with GM-CSF +IL-4 at the start of monocyte maturation the allogeneic response by PBMC is decreased to that of the background control.

These findings support the concept that BiP given before organ, tissue, cell, gene or protein administration will prevent immunisation. In terms of transplantation this should lead to acceptance of the graft.

Figure 10 shows that the PPD response by PBMC is significantly reduced to background levels when BiP is added to the PPD cultures.

These findings support the concept that the administration of BIP will suppress ongoing inflammatory and autoimmune diseases.

IN VIVO EXPERIMENTS

In developing BiP as a therapeutic drug two important properties need to be ascertained: first, the nature of the cytokines released after parenteral administration of BiP and, second, the optimum dose of BiP needed to achieve the maximum release of cytokines

1 BiP as an immunomodulator

Method: Male DBA-1 mice (8-12 weeks old) were immunised subcutaneously (s.c.) with 200 μg of BiP in phosphate buffered saline (PBS). PBS alone or bovine serum albumin (BSA) were administered as controls. 14 days later, spleens and lymph nodes were removed and T cell cultures set up and stimulated with varying concentrations of BiP at 0.1, 1, 10, 20 μg/ml. After 4 days of culture, the pro-inflammatory cytokine interferon (IFN)-γ and the anti-inflammatory cytokines interleukin (IL)-4, IL-5 and IL-10 were assayed. T cell proliferation was assessed by ^{3}H-thymidine incorporation.

Results: T cells derived from animals immunised with BiP proliferated in a dose dependent manner on *in vitro* stimulation (Figure 6, BiP). Control animals exhibited modest proliferation to *in vitro* stimulation with BiP (Figure 6, PBS). The cytokine profiles also demonstrated a dose dependent increase in IL-4 and IL-5 production by T cells derived from all BiP immunised mice (levels ~500 pg/ml and ~700 pg/ml respectively) (Figure 7) compared to the control animals (<100 pg/ml) (Figure 8). However, only modest levels of IFN-γ were produced by T cells derived from BiP-only immunised mice.

Conclusion: BiP is surprisingly immunogenic and immunising mice with BiP results in a raised anti-inflammatory cytokine profile with a switch towards a TH2 profile.

2 Optimising the BiP immunisation dose

Methods: Groups of mice were immunised with 4 different doses of BiP (either 50, 200, 500 μg or 1 mg), and 14 days later, spleen and lymph nodes were removed and the cells assayed for proliferation and cytokine secretion after 4 days in culture with BiP.

Results: It was evident from these experiments that the optimal immunising dose of BiP was 500 μg as T cells derived from mice re-stimulated *in vitro* with 10 μg/ml BiP proliferated most vigorously (600,000 CPM) whereas T cell proliferation from all other immunisation groups were below 40,000 CPM. Cytokines studies were in agreement showing levels of IL-4 reaching ~800 pg/ml in mice immunised with 500 μg (below 500 pg/ml in all other groups). Similarly IL-5 levels reached ~600 pg/ml in the 500 μg group and did not reach over 400 pg/ml in any of the other groups. T cells from naïve mice proliferated very slightly to BiP stimulation, produced very low quantities of Th2 cytokines and ~1000 pg/ml of IFN-γ.

Conclusion: The optimum proliferation and cytokine secretion upon *in vitro* BiP stimulation was observed when mice were immunised with 500 μg of BiP.

Claims

1. The use of BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, in the manufacture of a medicament for the treatment or prevention of an unwanted immune response.

2. The use according to claim 1, wherein the unwanted immune response is associated with an immune-mediated disease.

3. The use according to claim 2, wherein the immune-mediated disease is an auto-immune disease.

4. The use according to claim 2, wherein the immune-mediated disease is type 1 diabetes, thyroiditis or multiple sclerosis, systemic lupus erythematosus, Crohn's disease and all forms of viral and autoimmune hepatitis.

5. The use according to any one of claims 2 to 4, wherein the medicament additionally comprises an agent for enhancing the treatment or prevention of the immune-mediated disease.

6. The use according to any one of claims 2 to 5, wherein the medicament is administered to an individual suffering from or susceptible to developing an immune-mediated disease.

7. The use according to claim 6, wherein the medicament is administered to the individual prior to the development of the immune-mediated disease or as soon as the immune-mediated disease has be diagnosed.

8. The use according to claim 1, wherein the unwanted immune response is associated the rejection of a transplanted organ, a tissue or cells.

9. The use according to claim 8, wherein the medicament additionally comprises an agent for enhancing the treatment or prevention of transplant rejection.

10. The use according to claim 9, wherein the agent is a suppressor of the immune system.

11. The use according to any one of claims 8 to 10, wherein the medicament is administered to an individual before or at substantially the same time as the transplantation of an organ, tissue or cells.

12. The use according to claim 1, wherein the unwanted immune response is the immune response to a biologic.

13. The use according to claim 12, wherein the biologic is a therapeutic protein, glycoprotein or carbohydrate.

14. The use according to claim 12 or claim 13, wherein the medicament additionally comprises an agent for enhancing the treatment or prevention of the immune response to the biologic.

15. The use according to any one of claims 12 to 14, wherein the medicament is administered to an individual before or at substantially the same time as the biologic.

16. The use of BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, for stimulating the release of IL-10 from cells capable of releasing IL-10.

17. The use according to claim 16, wherein BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, additionally stimulates gene expression of at least one of monocyte migration inhibitory factor (MIP), soluble TNF receptor II, IL-10 anti-inflammatory mediators and tissue inhibitor of matrix metalloproteinases (TIMP).

18. The use according to claim 17, wherein BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, does not stimulate gene expression of matrix metalloproteinases (MMPs), monocyte chemoattractant protein (MCP-1) or TNFα.

19. A pharmaceutical preparation comprising BiP or a functional fragment or homolog thereof, or a nucleic acid molecule encoding BiP or a functional fragment or homolog thereof, in combination with a pharmaceutically acceptable carrier for use in the treatment or prevention of an unwanted immune response.

20. The pharmaceutical preparation according to claim 19, wherein the unwanted immune response is associated with an immune-mediated disease; associated with the rejection of a transplanted organ, tissue or cell; or is the immune response to a biologic.

21. The pharmaceutical composition according to claim 19 or claim 20, which additionally comprises an agent for enhancing the treatment of prevention of the unwanted immune response.

22. A method of treating or preventing an unwanted immune response comprising administrating to an individual in need of such treatment an effective dose of the pharmaceutical composition according to any one of claims 19 to 21.

23. The use of BiP or a peptide fragment thereof (including a synthetic peptide) for stimulating the release of Interleukin 10 (IL-10).

24. The use according to claim 23, for the preparation of a medicament for the treatment of auto-immune disease.

25. The use according to claim 24, for the preparation of a medicament for the prevention of rejection of transplanted organs , tissues, or cells.

26. The use according to claim 23, for the preparation of a medicament for the prevention of an immune response to biologic therapeutic substances.

27. The use according to claim 23, for the preparation of a medicament for the treatment of immune-mediated disease, including type 1 diabetes, thyroiditis, and multiple sclerosis, systemic lupus erythematosus, Crohn's disease and all forms of viral and autoimmune hepatitis.

28. Pharmaceutical preparations comprising BiP or a fragment thereof adapted for treatment of the diseases or situations referred to in any of claims 23 to 27.

29. A method of treatment of the diseases or situations referred to in any of claims 23 to 27, which comprises administering to a patient in need thereof BiP or a fragment thereof.

Fig. 1

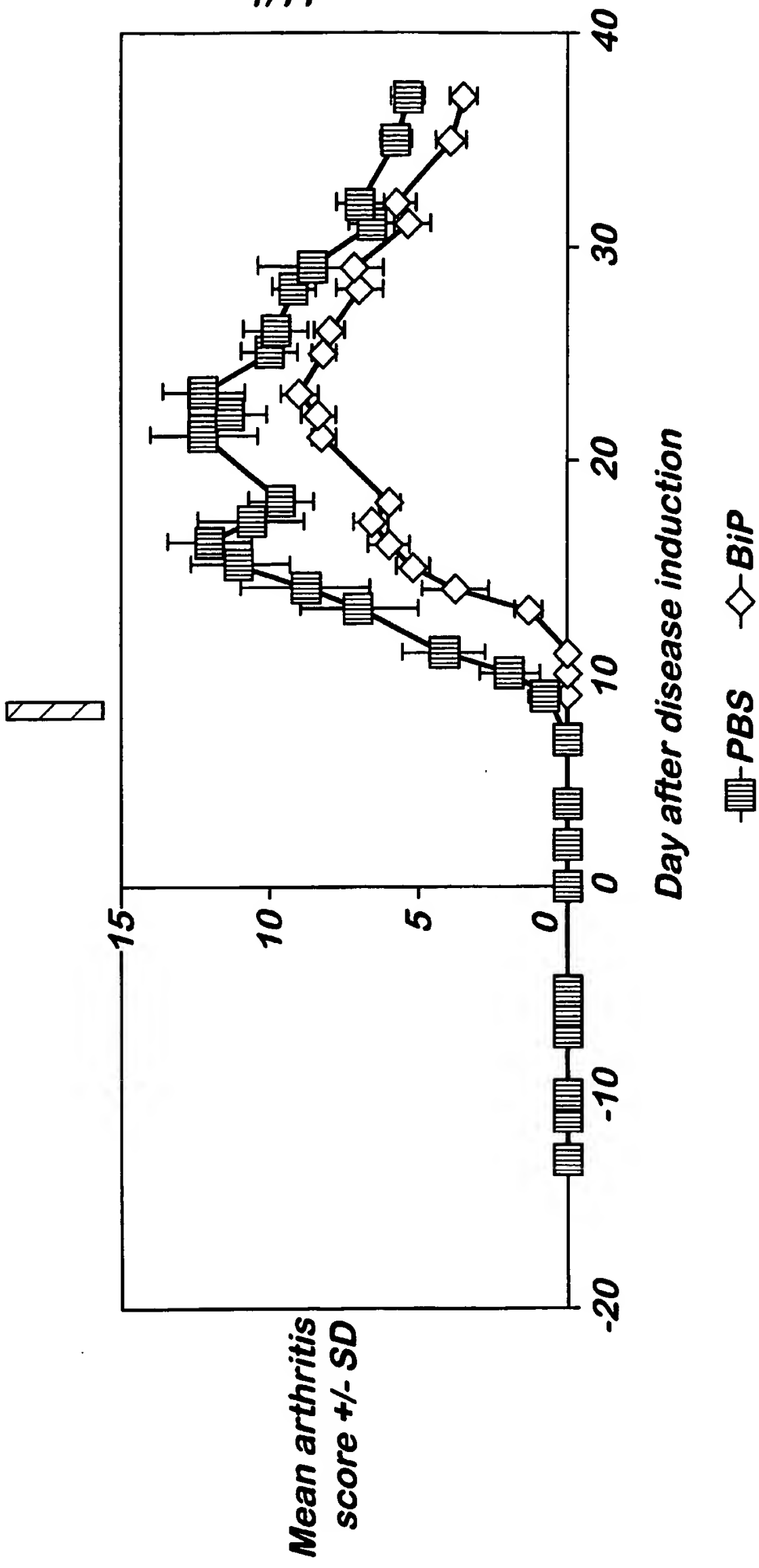

Mean arthritis score +/- SD
15
10
5
0
-20
-10
0
10
20
30
40
Day after disease induction
PBS
BiP

Fig. 2A

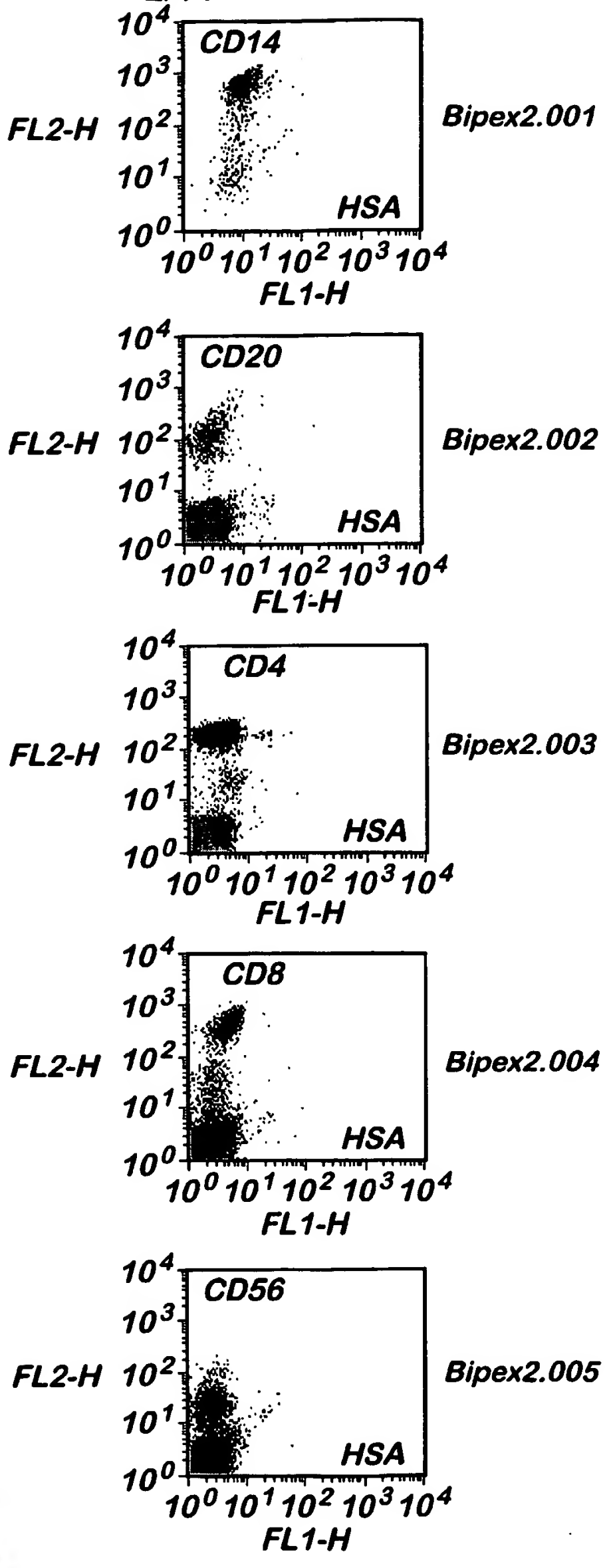

CD14
HSA
FL2-H
FL1-H
Bipex2.001
CD20
HSA
FL2-H
FL1-H
Bipex2.002
CD4
HSA
FL2-H
FL1-H
Bipex2.003
CD8
HSA
FL2-H
FL1-H
Bipex2.004
CD56
HSA
FL2-H
FL1-H
Bipex2.005

Fig. 2B

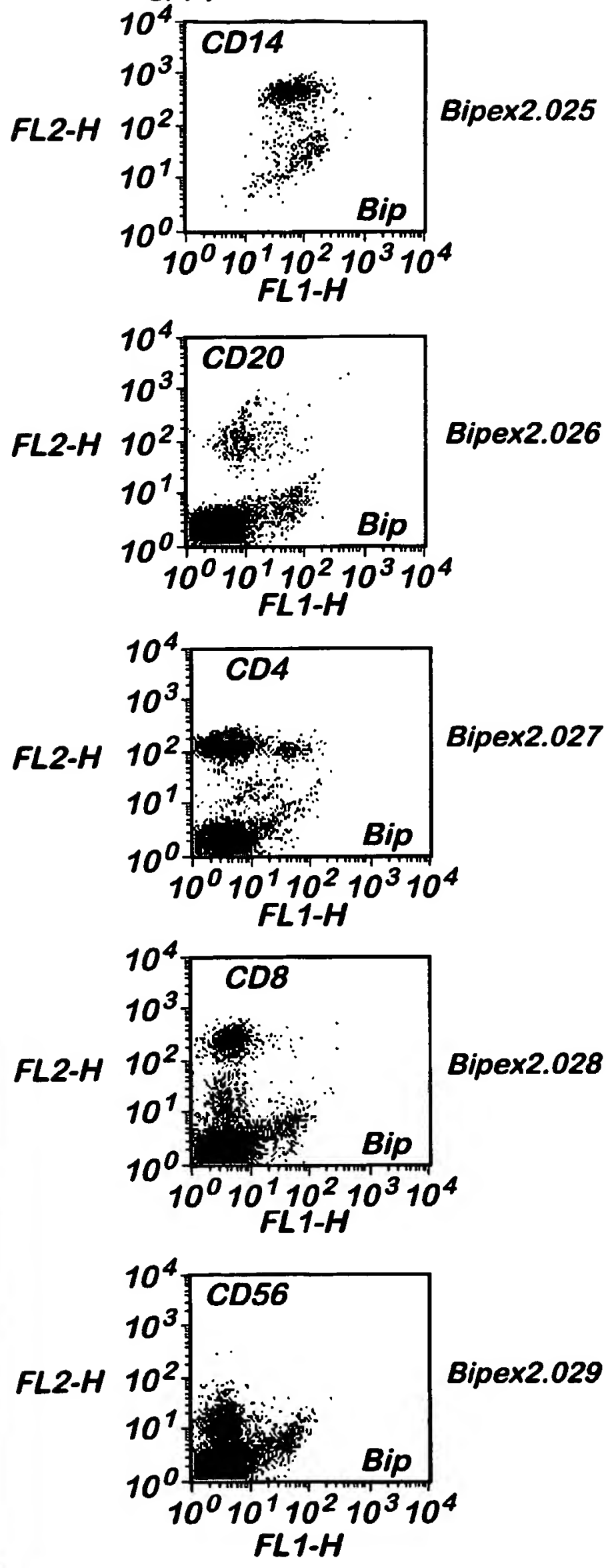

CD14
Bipex2.025
CD20
Bipex2.026
CD4
Bipex2.027
CD8
Bipex2.028
CD56
Bipex2.029
FL2-H
FL1-H
Bip
10^0 10^1 10^2 10^3 10^4

Fig. 2C

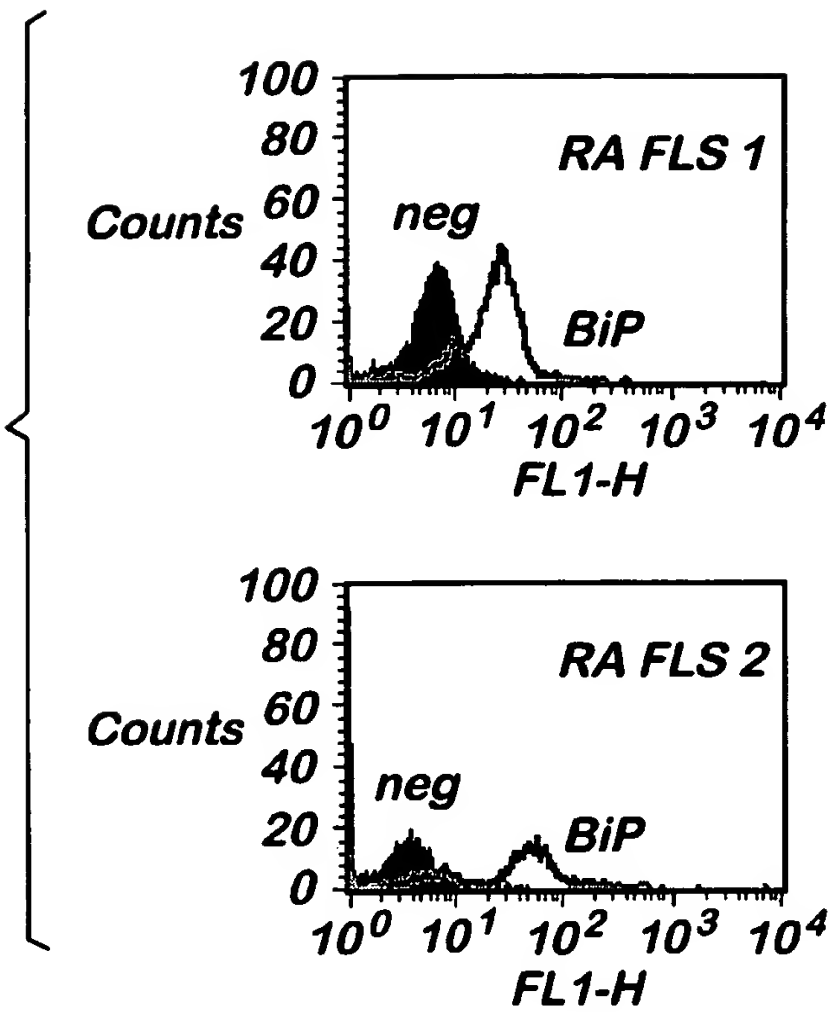

100
80
60
40
20
0
Counts
RA FLS 1
neg
BiP
10^0 10^1 10^2 10^3 10^4
FL1-H
100
80
60
40
20
0
Counts
RA FLS 2
neg
BiP
10^0 10^1 10^2 10^3 10^4
FL1-H

Fig. 3A

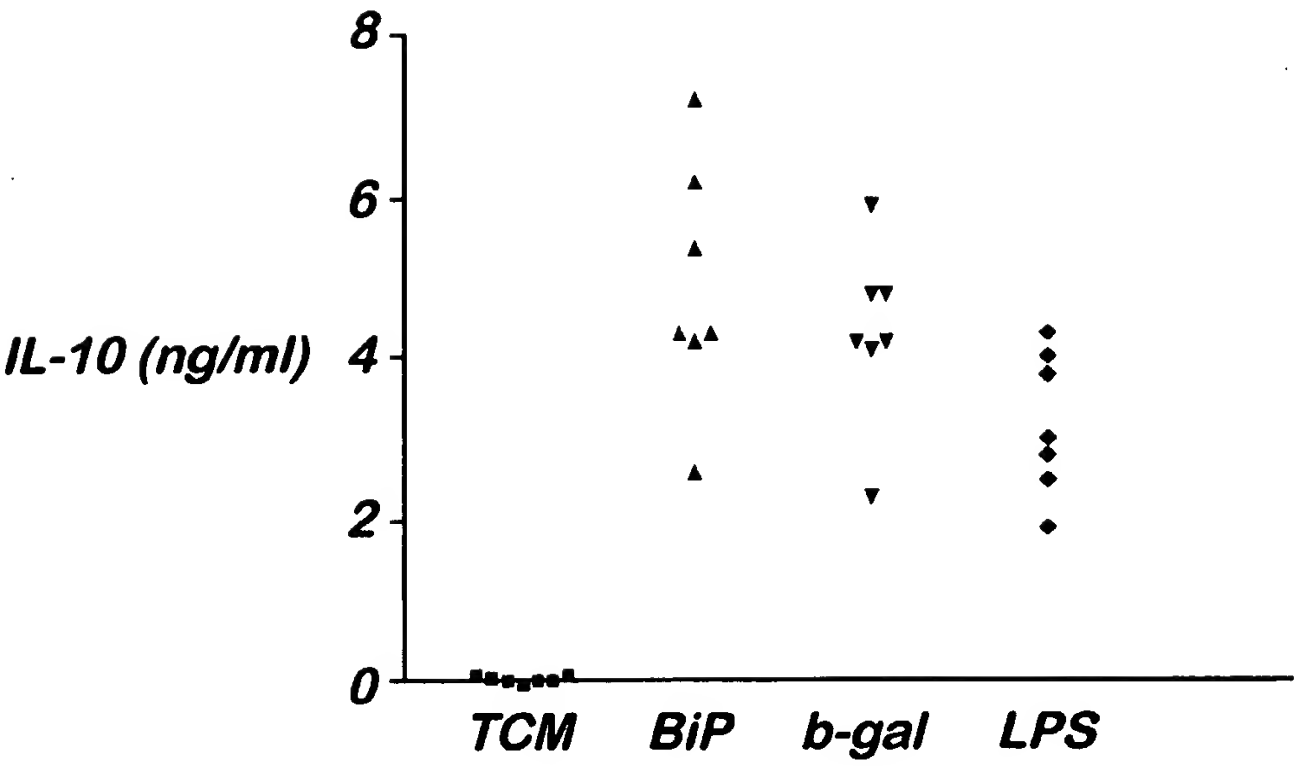
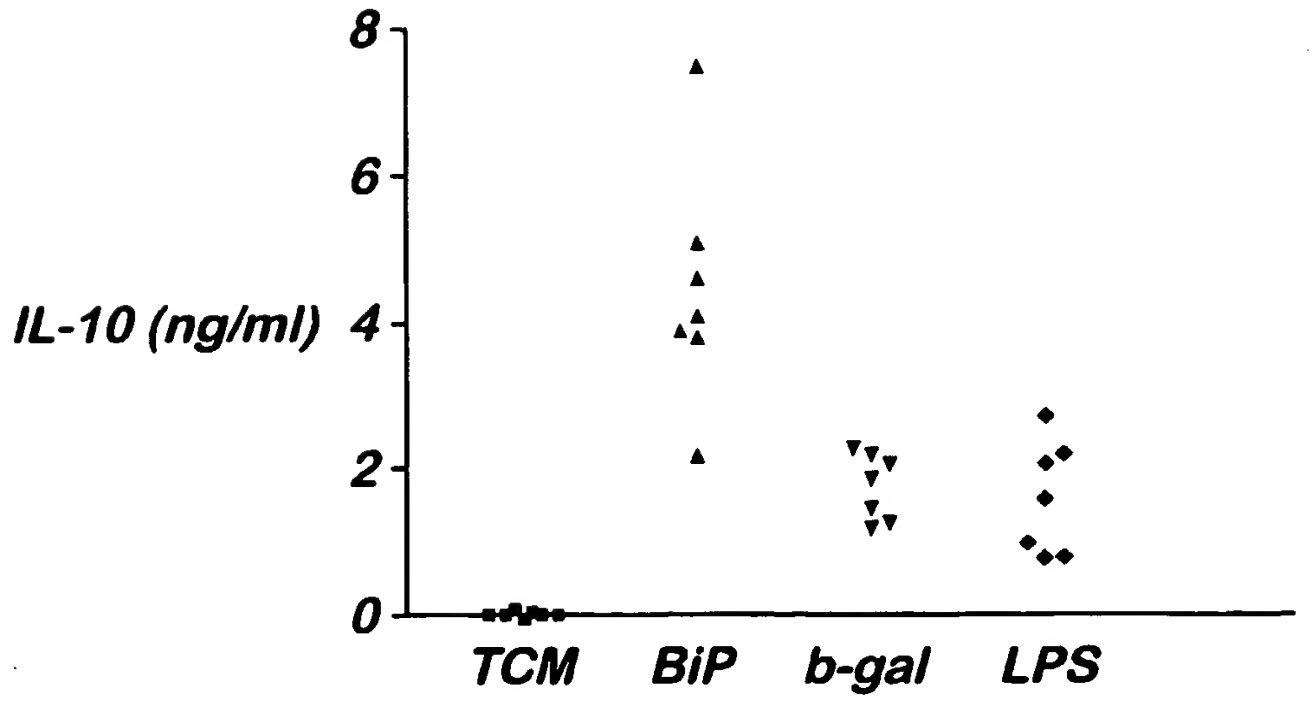

IL-10 (ng/ml)
8
6
4
2
0
TCM
BiP
b-gal
LPS

Fig. 3B


IL-10 (ng/ml)
8
6
4
2
0
TCM
BiP
b-gal
LPS

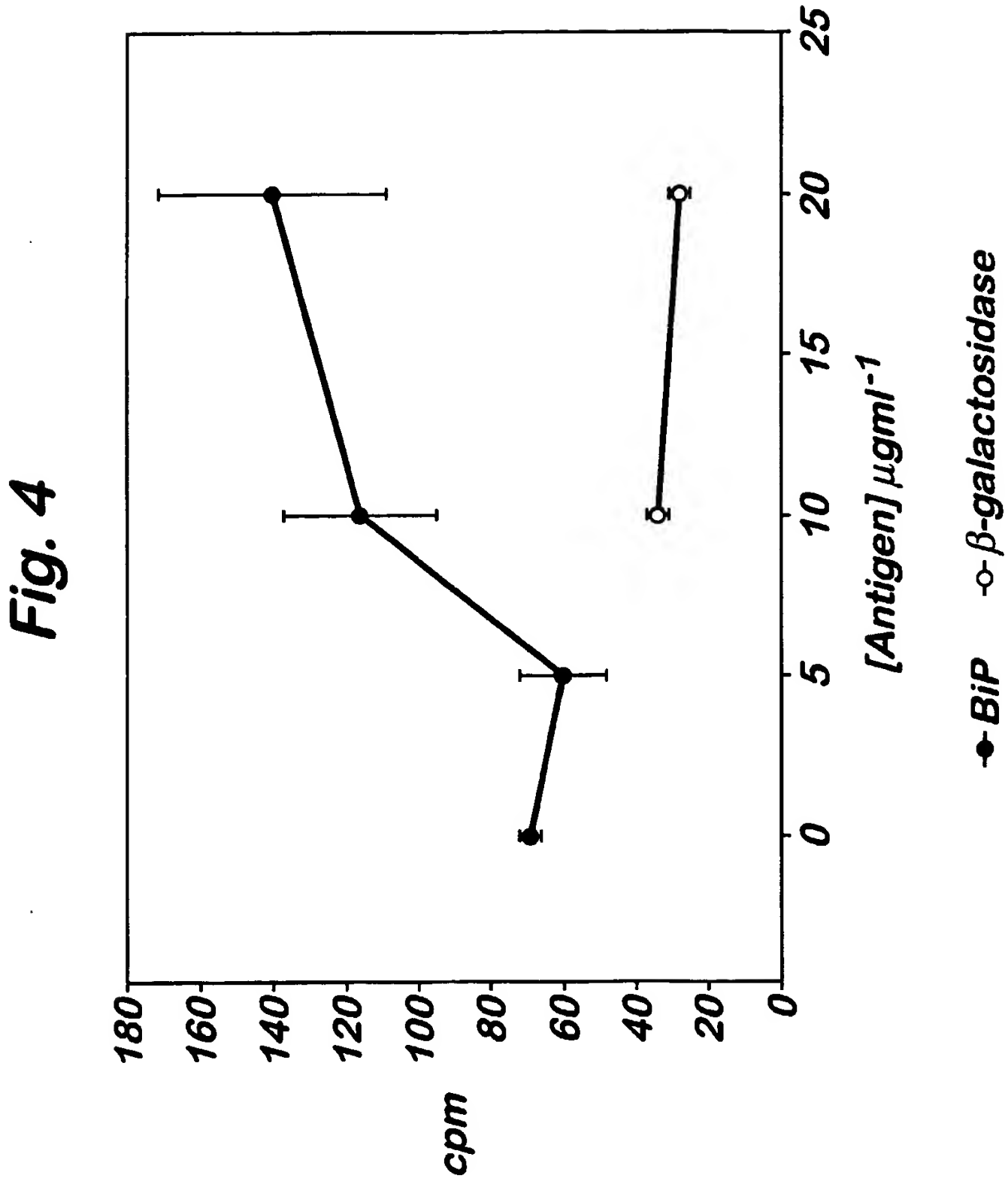

cpm
180
160
140
120
100
80
60
40
20
0
[Antigen] μgml-1
0
5
10
15
20
25
BiP
β-galactosidase

Fig. 4

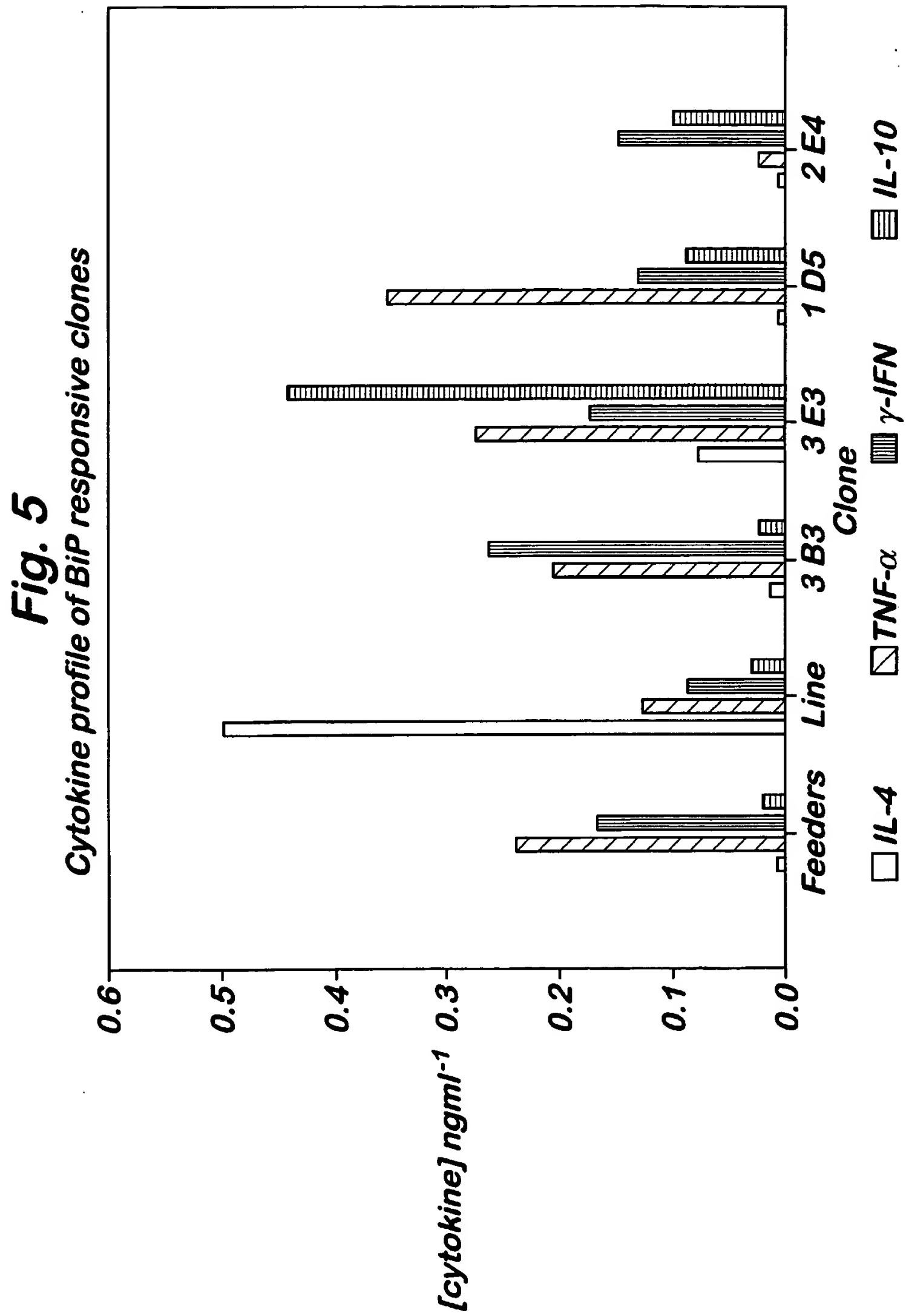


Fig. 5
Cytokine profile of BiP responsive clones
[cytokine] ngml-1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Feeders
Line
3 B3
3 E3
1 D5
2 E4
Clone
IL-4
TNF-α
γ-IFN
IL-10

Fig. 6

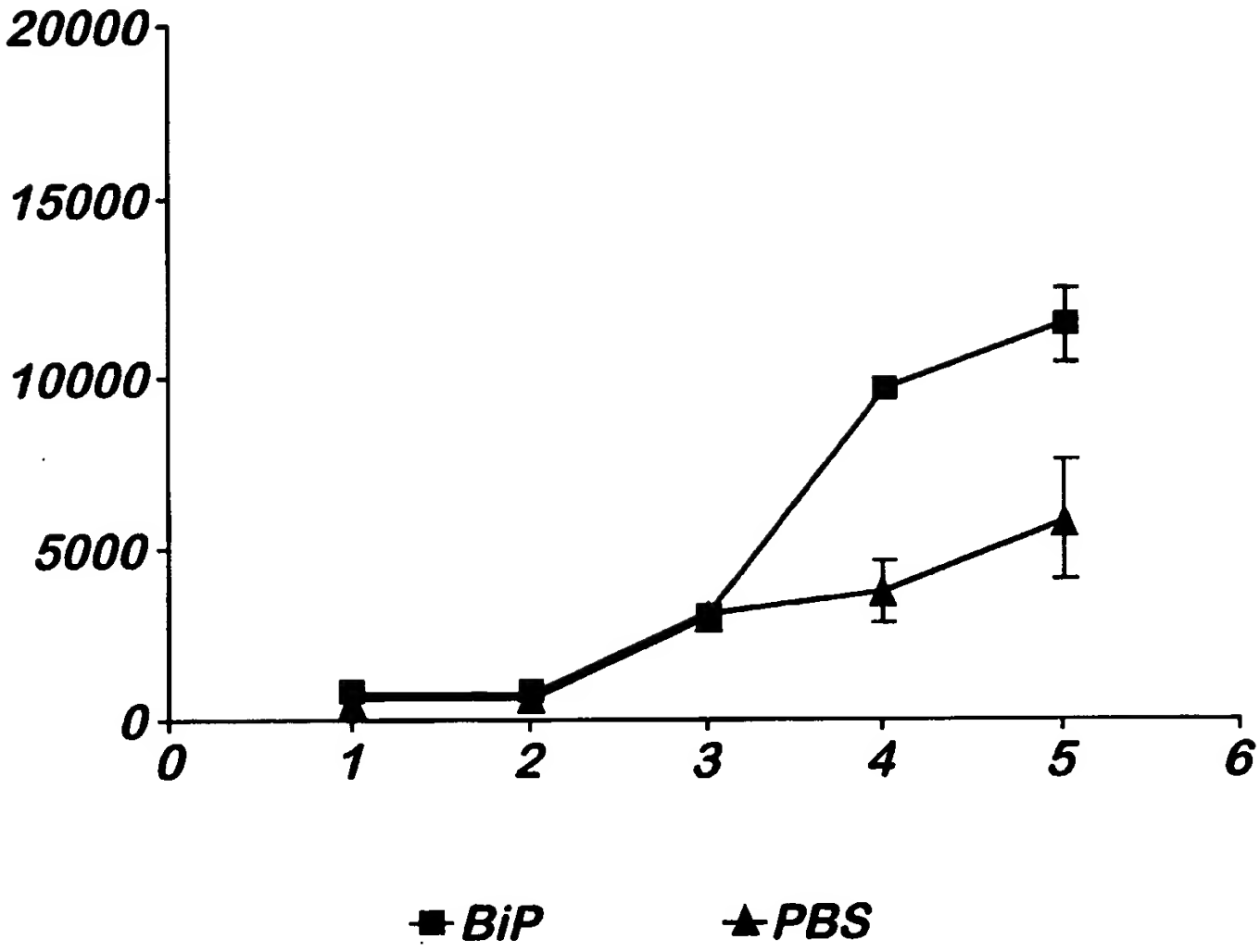
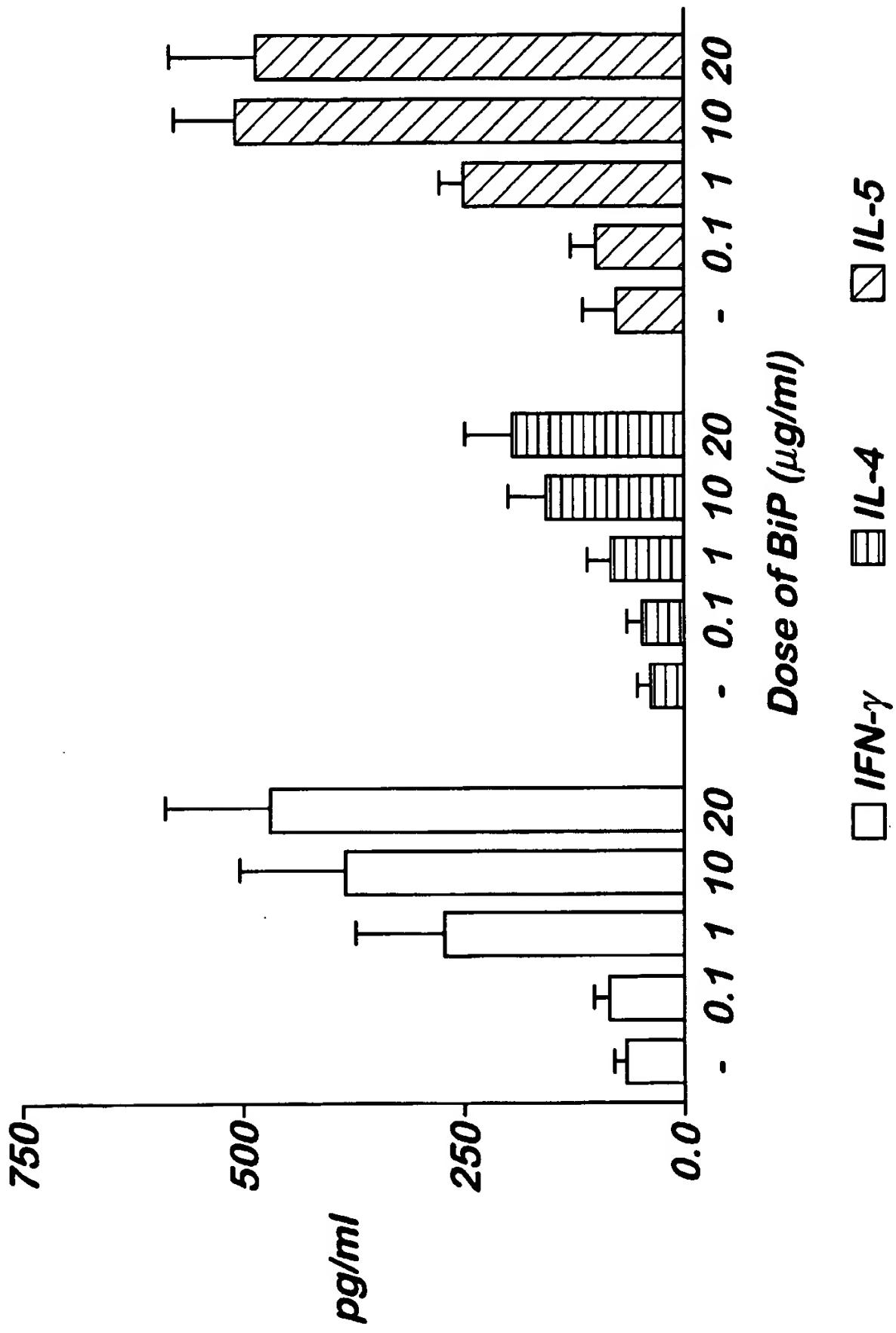


Fig. 7

BiP immunized animals



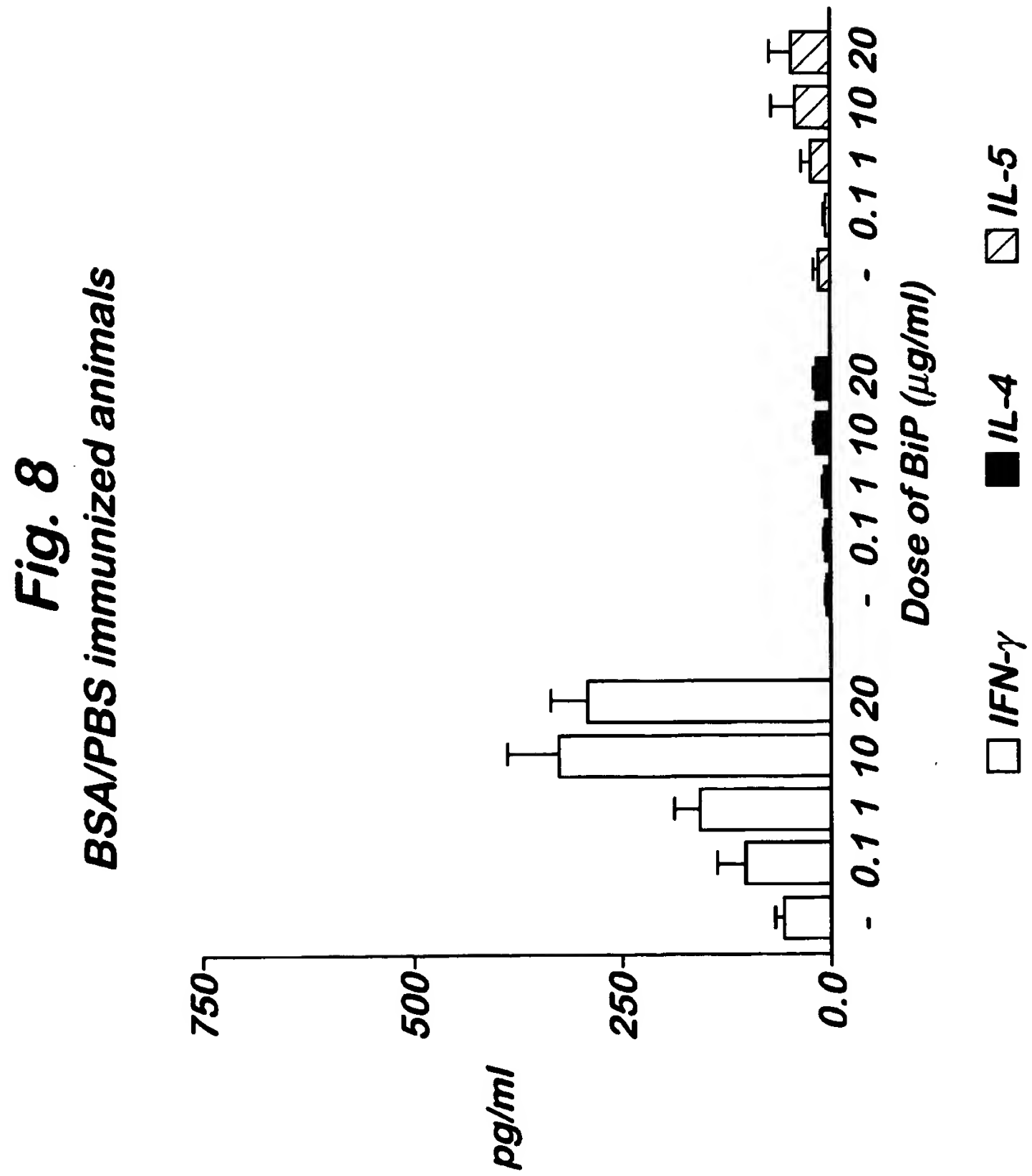


Fig. 8
BSA/PBS immunized animals
750
500
250
0.0
pg/ml
- 0.1 1 10 20
- 0.1 1 10 20
Dose of BiP (µg/ml)
IL-4
IL-5
IFN-γ

Fig. 9

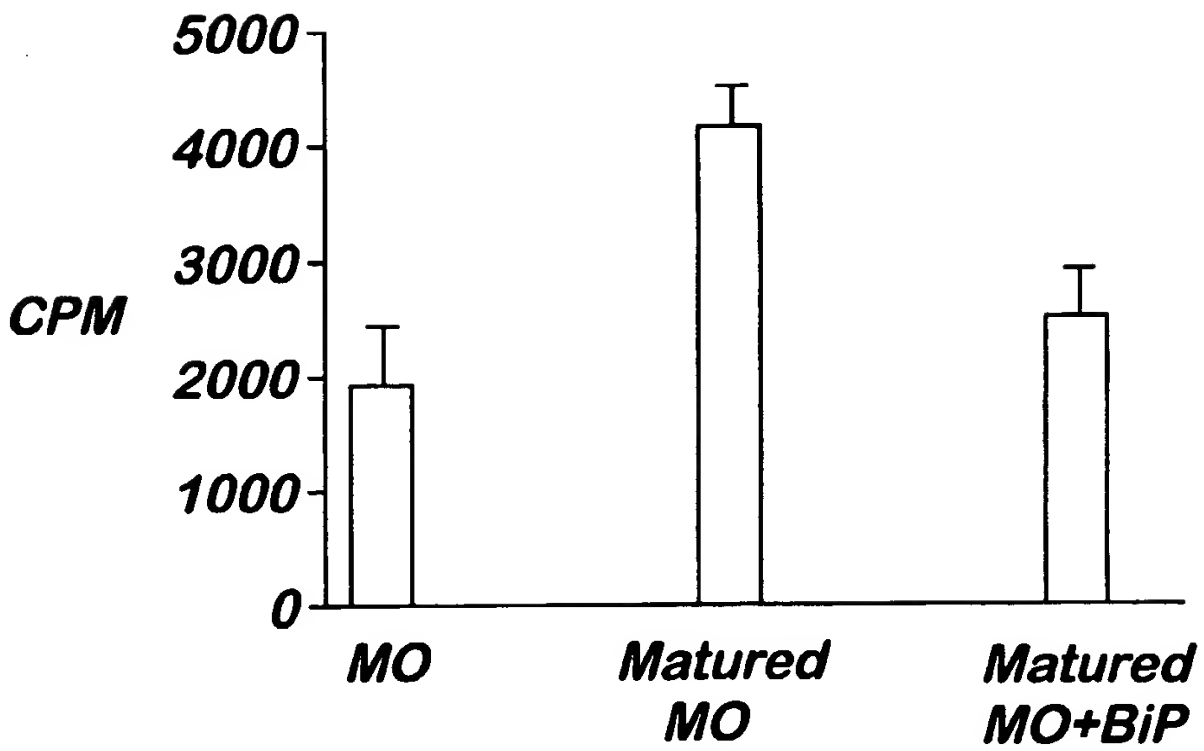
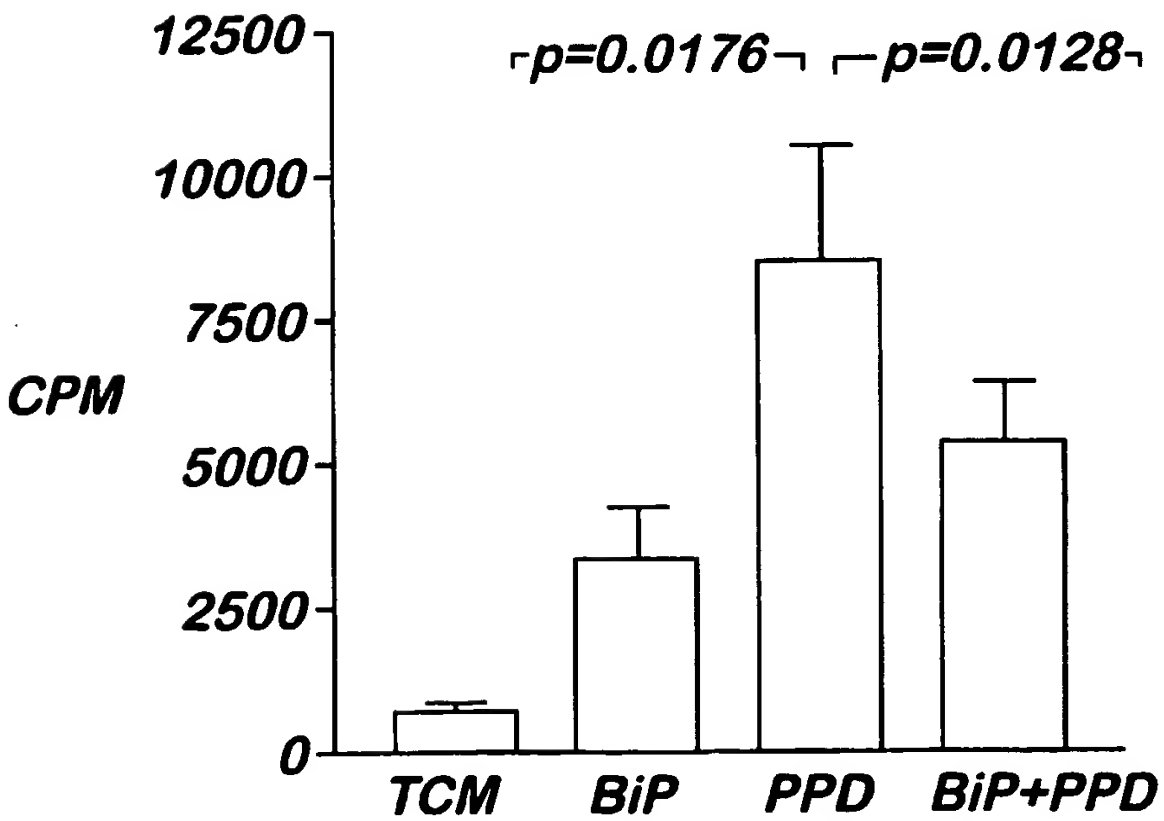

5000
4000
3000
2000
1000
0
CPM
MO
Matured MO
Matured MO+BiP

Fig. 10


12500
10000
7500
5000
2500
0
CPM
p=0.0176
p=0.0128
TCM
BiP
PPD
BiP+PPD

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/01151

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/17 A61P37/02 //(A61K38/17,31:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, PAJ, CHEM ABS Data, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 21995 A (FIFE MARK STEWART ;KING S COLLEGE LONDON (GB); BODMAN SMITH MARK D) 20 April 2000 (2000-04-20) cited in the application page 15, line 7 -page 16, line 25 page 17, line 27 -page 19, line 11 claims 8-11,15,16 --- -/--	1-3,5-7, 12-15, 19-22

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
3 June 2002	17/06/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stein, A

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INTERNATIONAL SEARCH REPORT

Ir tional Application No
PCT/GB 02/01151

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CORRIGALL VALERIE MARY ET AL: "A proposed immunoregulatory role for BiP through IL-10 production." FASEB JOURNAL, vol. 15, no. 5, 8 March 2001 (2001-03-08), page A1061 XP001080616 Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001;Orlando, Florida, USA; March 31-April 04, 2001 ISSN: 0892-6638 the whole document ---	16-18, 23-29
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INTERNATIONAL SEARCH REPORT

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		CA 2229595 A1	27-02-1997
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